updated search

(FILE 'HOME' ENTERED AT 15:30:55 ON 07 MAY 2003)

FILE 'USPATFULL' ENTERED AT 15:31:06 ON 07 MAY 2003

L1 2788 S NAPHTHOFLAVONE OR KAEMPFEROL OR CINNAMALDEHYDE OR LUTEOLIN
L2 2499 S NAPHTHOFLAVONE OR CINNAMALDEHYDE
L3 29 S (L2/CLM) AND L2/AB
L4 0 S L3 AND (CYTOCHROME P450)
L5 37 S L2 AND (CYTOCHROME P450)
L6 0 S L5 AND L3
L7 6 S L2 (2S) (CYTOCHROME P450)
L8 1 S L2 AND (CYTOCHROME P450 (1S) DERMAL)
L9 236 S ?NAPHTHOFLAVONE
L10 16 S L9/CLM

FILE 'CAPLUS, USPATFULL' ENTERED AT 16:03:45 ON 07 MAY 2003

L11 0 FILE CAPLUS
L12 0 FILE USPATFULL
TOTAL FOR ALL FILES
L13 0 S DERMAL CYTOCHROME P450
L14 19046 FILE CAPLUS
L15 1835 FILE USPATFULL
TOTAL FOR ALL FILES
L16 20881 S CYTOCHROME P450
L17 2 FILE CAPLUS
L18 3 FILE USPATFULL
TOTAL FOR ALL FILES
L19 5 S L16 (50A) DERMAL?
SAVE ALL L10079416/L
L20 0 FILE CAPLUS
L21 0 FILE USPATFULL
TOTAL FOR ALL FILES
L22 0 S (PHARMACEUTICAL COMPOSITION) (40A) (NAPHTHOFLAVONE)
L23 99 FILE CAPLUS
L24 4 FILE USPATFULL
TOTAL FOR ALL FILES
L25 103 S (COMPOSITION) (40A) (NAPHTHOFLAVONE)

FILE 'REGISTRY' ENTERED AT 17:06:47 ON 07 MAY 2003

L26 0 S NAPHTHOFLAVONE/CN
L27 16 S NAPHTHOFLAVONE
L28 10 S .ALPHA.- NAPHTHOFLAVONE

FILE 'CAPLUS' ENTERED AT 17:07:50 ON 07 MAY 2003

L29 17 S 604-59-1/THU

FILE 'CAPLUS' ENTERED AT 17:12:55 ON 07 MAY 2003

FILE 'USPATFULL' ENTERED AT 17:13:05 ON 07 MAY 2003

L30 1 S US6066642/PN
L31 1 S US6555523/PN
L32 1 S US5833994/PN
L33 1 S US6066642/PN
L34 3 S L31-33
L35 2 S (L29 OR L27 OR L28) AND L34

```
(FILE 'HOME' ENTERED AT 15:30:55 ON 07 MAY 2003)

     FILE 'USPATFULL' ENTERED AT 15:31:06 ON 07 MAY 2003
L1          2788 S NAPHTHOFLAVONE OR KAEMPFEROL OR CINNAMALDEHYDE OR LUTEOLIN
L2          2499 S NAPHTHOFLAVONE OR CINNAMALDEHYDE
L3            29 S (L2/CLM) AND L2/AB
L4             0 S L3 AND (CYTOCHROME P450)
L5            37 S L2 AND (CYTOCHROME P450)
L6             0 S L5 AND L3
L7             6 S L2 (2S) (CYTOCHROME P450)
L8             1 S L2 AND (CYTOCHROME P450 (1S) DERMAL)
L9           236 S ?NAPHTHOFLAVONE
L10           16 S L9/CLM

     FILE 'CAPLUS, USPATFULL' ENTERED AT 16:03:45 ON 07 MAY 2003
L11            0 FILE CAPLUS
L12            0 FILE USPATFULL
     TOTAL FOR ALL FILES
L13            0 S DERMAL CYTOCHROME P450
L14        19046 FILE CAPLUS
L15         1835 FILE USPATFULL
     TOTAL FOR ALL FILES
L16        20881 S  CYTOCHROME P450
L17            2 FILE CAPLUS
L18            3 FILE USPATFULL
     TOTAL FOR ALL FILES
L19            5 S L16 (50A) DERMAL?
                 SAVE ALL L10079416/L
L20            0 FILE CAPLUS
L21            0 FILE USPATFULL
     TOTAL FOR ALL FILES
L22            0 S (PHARMACEUTICAL COMPOSITION) (40A) (NAPHTHOFLAVONE)
L23           99 FILE CAPLUS
L24            4 FILE USPATFULL
     TOTAL FOR ALL FILES
L25          103 S (COMPOSITION) (40A) (NAPHTHOFLAVONE)

     FILE 'REGISTRY' ENTERED AT 17:06:47 ON 07 MAY 2003
L26            0 S NAPHTHOFLAVONE/CN
L27           16 S NAPHTHOFLAVONE
L28           10 S .ALPHA.- NAPHTHOFLAVONE

     FILE 'CAPLUS' ENTERED AT 17:07:50 ON 07 MAY 2003
L29           17 S 604-59-1/THU

     FILE 'CAPLUS' ENTERED AT 17:12:55 ON 07 MAY 2003

     FILE 'USPATFULL' ENTERED AT 17:13:05 ON 07 MAY 2003
L30            1 S US6066642/PN
L31            1 S US6555523/PN
L32            1 S US5833994/PN
L33            1 S US6066642/PN
L34            3 S L31-33
L35            2 S (L29 OR L27 OR L28) AND L34

=> save all
ENTER NAME OR (END):l10079416/l
'L10079416/L' IN USE
A single name cannot be used for two saved items at the same time.
Enter "Y" if you wish to replace the current saved name with a new
definition.  Enter "N" if the current saved definition must be
preserved.  You may then reenter the SAVE command with a different
saved name.  Enter "DISPLAY SAVED" at an arrow prompt (=>) to see a
list of your currently defined saved names.
```

REPLACE OLD DEFINITION? Y/(N):y
L# LIST L1-L35 HAS BEEN SAVED AS 'L10079416/L'

L29 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:133049 CAPLUS
DOCUMENT NUMBER: 138:163520
TITLE: Improved treatment of cancer with irinotecan based on genotyping of human gene UGT1A1 encoding UDP glycosyltransferase 1
INVENTOR(S): Heinrich, Guenther; Kerb, Reinhold
PATENT ASSIGNEE(S): Epidauros Biotechnologie AG, Germany
SOURCE: PCT Int. Appl., 107 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003013536	A2	20030220	WO 2002-EP8217	20020723

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2001-117608 A 20010723
EP 2002-11710 A 20020524

L29 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:587510 CAPLUS
DOCUMENT NUMBER: 138:214847
TITLE: Natural compounds with bronchodilator activity selected by molecular topology
AUTHOR(S): Rios-Santamarina, Imaculada; Garcia Domenech, Ramon; Cortijo, Julio; Santamaria, Pedro; Morcillo, Esteban J.; Galvez, Jorge
CORPORATE SOURCE: Unidad de Investigacion de Diseno de Farmacos y Conectividad Molecular, Departamento de Quimica-Fisica, Facultad de Farmacia, Universitat de Valencia, Burjassot, Valencia, 46100, Spain
SOURCE: Internet Electronic Journal of Molecular Design [online computer file] (2002), 1(2), 70-79
CODEN: IEJMAT; ISSN: 1538-6414
URL: http://biochempress.com/iejmd_2002_1_0070.pdf
PUBLISHER: BioChem Press
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:293387 CAPLUS
DOCUMENT NUMBER: 136:314998
TITLE: Compositions for alleviating adverse side effects and/or enhancing efficacy of agents inhibiting aromatase
INVENTOR(S): Kragie, Laura
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 34 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002030355	A2	20020418	WO 2001-US32066	20011010
WO 2002030355	A3	20030206		

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002013198	A5	20020422	AU 2002-13198	20011010

PRIORITY APPLN. INFO.:

US 2000-239457P	P	20001011
WO 2001-US32066	W	20011010

L29 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:142838 CAPLUS
DOCUMENT NUMBER: 136:177954
TITLE: Plant-derived and synthetic phenolic compounds and plant extracts, effective in the treatment and prevention of chlamydial infections
INVENTOR(S): Vuorela, Heikki; Vuorela, Pia; Hiltunen, Raimo; Leinonen, Maija; Saikku, Pekka
PATENT ASSIGNEE(S): Control-Ox Oy, Finland
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002014464	A2	20020221	WO 2001-FI726	20010816
WO 2002014464	A3	20020510		
WO 2002014464	B1	20021121		

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

FI 2000001832	A	20020219	FI 2000-1832	20000818
SG 90259	A1	20020723	SG 2001-4942	20010814
AU 2001082201	A5	20020225	AU 2001-82201	20010816

PRIORITY APPLN. INFO.:

US 2000-225735P	P	20000817
FI 2000-1832	A	20000818
WO 2001-FI726	W	20010816

L29 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:86479 CAPLUS
DOCUMENT NUMBER: 137:15388
TITLE: Flavonoids can block PSA production by breast and

prostate cancer cell lines

AUTHOR(S): Rosenberg Zand, Rachel S.; Jenkins, David J. A.; Brown, Theodore J.; Diamandis, Eleftherios P.

CORPORATE SOURCE: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Can.

SOURCE: Clinica Chimica Acta (2002), 317(1-2), 17-26
CODEN: CCATAR; ISSN: 0009-8981

PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 69 THERE ARE 69 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:436368 CAPLUS
DOCUMENT NUMBER: 135:268919
TITLE: Novel CFTR chloride channel activators identified by screening of combinatorial libraries based on flavone and benzoquinolizinium lead compounds

AUTHOR(S): Galietta, Luis J. V.; Springsteel, Mark F.; Eda, Masahiro; Niedzinski, Edmund J.; By, Kolbot; Haddadin, M. J.; Kurth, Mark J.; Nantz, Michael H.; Verkman, A. S.

CORPORATE SOURCE: Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, CA, 94143-0521, USA

SOURCE: Journal of Biological Chemistry (2001), 276(23), 19723-19728
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:321522 CAPLUS
DOCUMENT NUMBER: 135:175237
TITLE: Protection against nitrofurantoin-induced oxidative stress by coelenterazine analogues and their oxidation products in rat hepatocytes

AUTHOR(S): Dubuisson, Marlene L. N.; De Wergifosse, Bertrand; Kremers, Pierre; Marchand-Brynaert, Jacqueline; Trouet, Andre; Rees, Jean-Francois

CORPORATE SOURCE: Unite de Biologie Animale, Universite Catholique de Louvain, Louvain-la-Neuve, B-1348, Belg.

SOURCE: Free Radical Research (2001), 34(3), 285-296
CODEN: FRARER; ISSN: 1071-5762

PUBLISHER: Harwood Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:50468 CAPLUS
DOCUMENT NUMBER: 134:110442
TITLE: Use of flavones, coumarins and related compounds to treat infections

INVENTOR(S): Prendergast, Patrick T.
PATENT ASSIGNEE(S): Ire.
SOURCE: PCT Int. Appl., 70 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001003681	A2	20010118	WO 2000-IB1039	20000707
WO 2001003681	A3	20020510		

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1223928	A2	20020724	EP 2000-948187	20000707
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
JP 2003504327	T2	20030204	JP 2001-508962	20000707
US 6555523	B1	20030429	US 2000-612025	20000707

PRIORITY APPLN. INFO.: US 1999-142894P P 19990708
US 1999-163089P P 19991102
WO 2000-IB1039 W 20000707

OTHER SOURCE(S): MARPAT 134:110442

L29 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:536399 CAPLUS
DOCUMENT NUMBER: 131:281114
TITLE: Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor
AUTHOR(S): Reiners, John J., Jr.; Clift, Russell; Mathieu, Patricia
CORPORATE SOURCE: Institute of Chemical Toxicology, Wayne State University, Detroit, MI, 48201, USA
SOURCE: Carcinogenesis (1999), 20(8), 1561-1566
CODEN: CRNGDP; ISSN: 0143-3334
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:534825 CAPLUS
DOCUMENT NUMBER: 131:317407
TITLE: Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats
AUTHOR(S): Mori, Hideki; Sugie, Shigeyuki; Rahman, Wahidor; Suzui, Natsuko
CORPORATE SOURCE: Department of Pathology, Gifu University School of Medicine, Tsukasa-machi, Gifu, Japan
SOURCE: Cancer Letters (Shannon, Ireland) (1999), 143(2), 195-198
CODEN: CALEDQ; ISSN: 0304-3835
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:311102 CAPLUS
DOCUMENT NUMBER: 130:332910
TITLE: Methods and compositions for regulation of 5-alpha reductase activity
INVENTOR(S): Liao, Shutsung; Hiipakka, Richard A.
PATENT ASSIGNEE(S): Arch Development Corporation, USA
SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9922728	A1	19990514	WO 1998-US23041	19981030
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9912898	A1	19990524	AU 1999-12898	19981030
EP 1027045	A1	20000816	EP 1998-956358	19981030
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, SE, PT, IE				

PRIORITY APPLN. INFO.: US 1997-63770P P 19971031
WO 1998-US23041 W 19981030
OTHER SOURCE(S): MARPAT 130:332910
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:130584 CAPLUS
DOCUMENT NUMBER: 130:200924
TITLE: Compositions and treatments to reduce side effects of administration of androgenic testosterone precursors
PATENT ASSIGNEE(S): Weider Nutrition International, Inc., USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9907381	A1	19990218	WO 1998-US16679	19980811
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9887798	A1	19990301	AU 1998-87798	19980811

PRIORITY APPLN. INFO.: US 1997-55346P P 19970811
WO 1998-US16679 W 19980811
REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:490511 CAPLUS
DOCUMENT NUMBER: 129:131240
TITLE: Use of the aryl hydrocarbon (Ah) receptor and Ah receptor ligands to treat or prevent the cytopathicity of viral infection
INVENTOR(S): Wheelock, Geoffrey D.; Rininger, Joseph; Babish, John G.; Chigurupati, Padmasree
PATENT ASSIGNEE(S): Paracelsian, Inc., USA
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9830213	A2	19980716	WO 1998-US139	19980107
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5833994	A	19981110	US 1997-780742	19970108
AU 9857321	A1	19980803	AU 1998-57321	19980107
US 6140063	A	20001031	US 1998-294442	19980813

PRIORITY APPLN. INFO.: US 1997-780742 A 19970108
WO 1998-US139 W 19980107

L29 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:513625 CAPLUS
DOCUMENT NUMBER: 127:190650
TITLE: Preparation of dihydropyridines, pyridines, benzopyranones, and triazoloquinazolines for use as adenosine receptor antagonists
INVENTOR(S): Jacobson, Kenneth A.; Jiang, Ji-Long; Kim, Yong-Chul; Karton, Yishai; Van Rhee, Albert M.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; Jacobson, Kenneth A.; Jiang, Ji-Long; Kim, Yong-Chul; Karton, Yishai; Van Rhee, Albert M.
SOURCE: PCT Int. Appl., 138 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9727177	A2	19970731	WO 1997-US1252	19970129
WO 9727177	A3	19971113		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				

CA 2244774	AA	19970731	CA 1997-2244774	19970129
AU 9722466	A1	19970820	AU 1997-22466	19970129
AU 709190	B2	19990826		
EP 885192	A1	19981223	EP 1997-905627	19970129
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000516910	T2	20001219	JP 1997-527065	19970129
US 6066642	A	20000523	US 1998-117598	19981207
AU 755525	B2	20021212	AU 1999-57171	19991101
AU 9957171	A1	20000217		

PRIORITY APPLN. INFO.: US 1996-10737P P 19960129
US 1996-21191P P 19960703
WO 1997-US1252 W 19970129

OTHER SOURCE(S): MARPAT 127:190650

L29 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:113497 CAPLUS
DOCUMENT NUMBER: 126:207197
TITLE: Differential mechanisms of cytochrome P450 inhibition and activation by .alpha.-naphthoflavone
AUTHOR(S): Koley, Aditya P.; Buters, Jeroen T. M.; Robinson, Richard C.; Markowitz, Allen; Friedman, Fred K.
CORPORATE SOURCE: Laboratory of Molecular Carcinogenesis, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Journal of Biological Chemistry (1997), 272(6), 3149-3152
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

L29 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:32789 CAPLUS
DOCUMENT NUMBER: 124:164289
TITLE: Interactions of Flavonoids and Other Phytochemicals with Adenosine Receptors
AUTHOR(S): Ji, Xiao-duo; Melman, Neli; Jacobson, Kenneth A.
CORPORATE SOURCE: Laboratory of Bioorganic Chemistry, National Institute of Diabetes Digestive and Kidney Diseases, Bethesda, MD, 20892-0810, USA
SOURCE: Journal of Medicinal Chemistry (1996), 39(3), 781-8
CODEN: JMCMAR; ISSN: 0022-2623
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

L29 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1979:503407 CAPLUS
DOCUMENT NUMBER: 91:103407
TITLE: Ellipticines as potent inhibitors of microsomes-dependent chemical mutagenesis
AUTHOR(S): Lesca, P.; Lecointe, P.; Paoletti, C.; Mansuy, D.
CORPORATE SOURCE: Lab. Pharmacol. Toxicol. Fondam., Toulouse, 31078, Fr.
SOURCE: Chemico-Biological Interactions (1979), 25(2-3), 279-87
CODEN: CBINA8; ISSN: 0009-2797
DOCUMENT TYPE: Journal
LANGUAGE: English

=>

loxy; when R.sub.1, R.sub.2, and R.sub.3 are hydrogen, R.sub.4 is neither phenyl nor alkyloxyphenyl; and when R.sub.3 is hydrogen and R.sub.4 is phenyl, neither R.sub.1 nor R.sub.2 is alkylcarbonyloxy.

23. A method of treating a mammal comprising selectively blocking one or more adenosine receptors of said mammal by administering to said mammal at least one compound selected from the group consisting of genistein, (.+-.)dihydrogenistein, sakuranetin, .alpha.-**naphthoflavone**, .beta.-**naphthoflavone**, amaryllidaceae, oxogalanthine lactam, acetylhaemanthine methiodide, 2,3-methylenedioxy-fluorene-9-one, hematoxylin, and arborinine.

24. A compound of the formula ##STR41## or a pharmaceutically acceptable salt thereof, wherein R.sub.1 is selected from the group consisting of C.sub.1 -C.sub.6 alkylcarbonyl, aryl C.sub.1 -C.sub.6 alkylcarbonyl, aryl C.sub.2 -C.sub.6 alkenylcarbonyl, C.sub.1 -C.sub.6 alkyloxycarbonyl, amino C.sub.1 -C.sub.6 alkylcarbonyl, and arylcarbonyl, wherein said aryl may be further substituted with halo, nitro, hydroxy, amino or cyano; and R.sub.2 is hydrogen or halogen.

25. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of claim 19.

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of claim 20.

27. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of claim 21.

28. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of claim 24.

29. A method of treating a mammal comprising selectively blocking an adenosine receptor of a mammal by administering to said mammal a compound of claim 19.

30. A method of treating a mammal comprising selectively blocking an adenosine receptor of a mammal by administering to said mammal a compound of claim 20.

31. A method of treating a mammal comprising selectively blocking an adenosine receptor of a mammal by administering to said mammal a compound of claim 21.

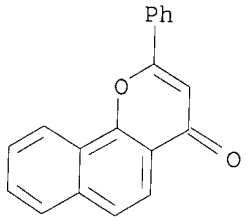
32. A method of treating a mammal comprising selectively blocking an adenosine receptor of a mammal by administering to said mammal a compound of claim 24.

33. A method of cerebroprotecting a mammal comprising selectively blocking the A.sub.3 adenosine receptor of the mammal by administering to the mammal an effective amount of a compound of claim 19.

PI US 6066642 20000523
WO 9727177 19970731

=>

L28 ANSWER 10 OF 10 REGISTRY COPYRIGHT 2003 ACS
RN 604-59-1 REGISTRY
CN 4H-Naphtho[1,2-b]pyran-4-one, 2-phenyl- (8CI, 9CI) (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN 7,8-Benzoflavone (6CI, 7CI)
OTHER NAMES:
CN **.alpha.-Naphthoflavone**
CN .alpha.-Naphthylflavone
CN ANF
CN Benzo[h]flavone
CN UCCF 023
FS 3D CONCORD
MF C19 H12 O2
CI COM
LC STN Files: AGRICOLA, ANABSTR, AQUIRE, BEILSTEIN*, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CHEMCATS, CHEMINFORMRX, CHEMLIST, CSCHEM, DDFU, DRUGU, EMBASE, HODOC*, IFICDB, IFIPAT, IFIUDB, MEDLINE, MSDS-OHS, NIOSHTIC, PROMT, RTECS*, SPECINFO, TOXCENTER, ULIDAT, USPATFULL
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

720 REFERENCES IN FILE CA (1957 TO DATE)
11 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
721 REFERENCES IN FILE CAPLUS (1957 TO DATE)
9 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

=>

L29 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2003 ACS

IT **604-59-1**, .alpha.-Naphthoflavone
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); **THU (Therapeutic use)**; BIOL (Biological study); USES (Uses)
(mechanisms of cytochrome P 450 inhibition and activation by .alpha.-naphthoflavone)

ACCESSION NUMBER: 1997:113497 CAPLUS
DOCUMENT NUMBER: 126:207197
TITLE: Differential mechanisms of cytochrome P450 inhibition and activation by .alpha.-naphthoflavone
AUTHOR(S): Koley, Aditya P.; Buters, Jeroen T. M.; Robinson, Richard C.; Markowitz, Allen; Friedman, Fred K.
CORPORATE SOURCE: Laboratory of Molecular Carcinogenesis, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Journal of Biological Chemistry (1997), 272(6), 3149-3152
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

L29 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2003 ACS
IT 446-72-0, Genistein 487-26-3, Flavanone 491-78-1 517-28-2, Hema-toxylin 520-36-5, Apigenin 525-82-6, Flavone **604-59-1**, .alpha.-Naphthoflavone 1447-88-7, Hispidulin 2957-21-3, Sakuranetin 5128-44-9 5938-16-9 6051-87-2, .beta.-Naphthoflavone 6601-62-3, Cirsimaritin 6665-86-7 13178-98-8 16692-52-7, Tetramethylkaempferol 17348-76-4 21829-25-4, Nifedipine 33500-23-1 33513-36-9 55985-32-5 66085-59-4 67035-22-7 102993-22-6 123180-08-5 173788-52-8, 4',5,6,7-Tetramethylscutallarein 173788-53-9, Acetylhaemanthamine methoidide 176220-94-3 183721-12-2 183721-16-6 185222-66-6 185222-67-7 185222-70-2 185222-74-6 185222-76-8 185222-77-9 185222-79-1 185222-80-4 185222-81-5 185222-82-6 185222-83-7 185222-84-8 185222-86-0 185222-87-1 185222-88-2 185222-89-3 185222-90-6 185223-17-0 192052-93-0 192052-95-2 192053-02-4 192053-03-5 192053-09-1 192053-17-1 192053-20-6 192053-23-9 194346-55-9 194346-77-5 194346-96-8 194346-97-9 194346-98-0 194346-99-1 194347-00-7 194347-03-0 194347-04-1 194347-05-2 194347-06-3 194347-07-4 194347-10-9 194347-11-0 194347-12-1 194347-13-2 194347-14-3 194347-16-5 194347-17-6 194347-18-7
RL: BAC (Biological activity or effector, except adverse); **THU (Therapeutic use)**; BSU (Biological study, unclassified); BIOL (Biological study); USES (Uses)
(prepn. of dihydropyridines, pyridines, benzopyranones, and triazoloquinazolines for use as adenosine receptor antagonists)

ACCESSION NUMBER: 1997:513625 CAPLUS
DOCUMENT NUMBER: 127:190650
TITLE: Preparation of dihydropyridines, pyridines, benzopyranones, and triazoloquinazolines for use as adenosine receptor antagonists
INVENTOR(S): Jacobson, Kenneth A.; Jiang, Ji-Long; Kim, Yong-Chul; Karton, Yishai; Van Rhee, Albert M.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; Jacobson, Kenneth A.; Jiang, Ji-Long; Kim, Yong-Chul; Karton, Yishai; Van Rhee, Albert M.
SOURCE: PCT Int. Appl., 138 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9727177	A2	19970731	WO 1997-US1252	19970129
WO 9727177	A3	19971113		
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2244774	AA	19970731	CA 1997-2244774	19970129
AU 9722466	A1	19970820	AU 1997-22466	19970129
AU 709190	B2	19990826		
EP 885192	A1	19981223	EP 1997-905627	19970129
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000516910	T2	20001219	JP 1997-527065	19970129
US 6066642	A	20000523	US 1998-117598	19981207
AU 755525	B2	20021212	AU 1999-57171	19991101
AU 9957171	A1	20000217		

PRIORITY APPLN. INFO.: US 1996-10737P P 19960129
US 1996-21191P P 19960703

L29 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2003 ACS

IT 508-02-1, Oleanolic acid **604-59-1**, .alpha.-Naphthoflavone 1746-01-6, 2,3,7,8-Tetrachlorodibenzo-p-dioxin 5508-58-7, Andrographolide 32598-13-3, 3,3',4,4'-Tetrachlorobiphenyl 39227-28-6, 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin 39227-54-8, 2-Monochlorodibenzo-p-dioxin 51207-31-9, 2,3,7,8-TCDF 57465-28-8, 3,3',4,4',5-Pentachlorobiphenyl

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); **THU (Therapeutic use)**; BIOL (Biological study); USES (Uses)

(aryl hydrocarbon (Ah) receptor and Ah receptor ligands and other compds. to treat or prevent cytopathicity of viral infection)

ACCESSION NUMBER: 1998:490511 CAPLUS

DOCUMENT NUMBER: 129:131240

TITLE: Use of the aryl hydrocarbon (Ah) receptor and Ah receptor ligands to treat or prevent the cytopathicity of viral infection

INVENTOR(S): Wheelock, Geoffrey D.; Rininger, Joseph; Babish, John G.; Chigurupati, Padmasree

PATENT ASSIGNEE(S): Paracelsian, Inc., USA

SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9830213	A2	19980716	WO 1998-US139	19980107
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5833994	A	19981110	US 1997-780742	19970108
AU 9857321	A1	19980803	AU 1998-57321	19980107
US 6140063	A	20001031	US 1998-294442	19980813

PRIORITY APPLN. INFO.:

L29 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2003 ACS
IT 520-36-5, Apigenin 525-82-6, Flavone **604-59-1**, .alpha.-Naphthoflavone 145370-39-4, 3'-Methoxy-4'-nitroflavone 167869-21-8, PD98059)
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); **THU (Therapeutic use)**; BIOL (Biological study); USES (Uses)
(flavonoids suppression of cell cycle progression: dependence on aryl hydrocarbon receptor)

ACCESSION NUMBER: 1999:536399 CAPLUS
DOCUMENT NUMBER: 131:281114
TITLE: Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor
AUTHOR(S): Reiners, John J., Jr.; Clift, Russell; Mathieu, Patricia
CORPORATE SOURCE: Institute of Chemical Toxicology, Wayne State University, Detroit, MI, 48201, USA
SOURCE: Carcinogenesis (1999), 20(8), 1561-1566
CODEN: CRNGDP; ISSN: 0143-3334
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2003 ACS
IT 50-78-2, Aspirin **604-59-1**, .alpha.-Naphthoflavone 2179-57-9, Diallyl disulfide 6051-87-2, .beta.-Naphthoflavone
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); **THU (Therapeutic use)**; BIOL (Biological study); USES (Uses)
(chemoprevention of PhIP-induced mammary carcinogenesis in rats with dietary)

ACCESSION NUMBER: 1999:534825 CAPLUS
DOCUMENT NUMBER: 131:317407
TITLE: Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats
AUTHOR(S): Mori, Hideki; Sugie, Shigeyuki; Rahman, Wahidor; Suzui, Natsuko
CORPORATE SOURCE: Department of Pathology, Gifu University School of Medicine, Tsukasa-machi, Gifu, Japan
SOURCE: Cancer Letters (Shannon, Ireland) (1999), 143(2), 195-198
CODEN: CALEDQ; ISSN: 0304-3835
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 1998:604278 CAPLUS
DN 129:286850
TI Disposition of butanal oxime in rat following oral, intravenous and dermal administration
AU Mathews, J. M.; Black, S. R.; Burka, L. T.
CS Center for Bioorganic Chemistry, Research Triangle Institute and t National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709, USA
SO Xenobiotica (1998), 28(8), 767-777
CODEN: XENOBH; ISSN: 0049-8254
PB Taylor & Francis Ltd.
DT Journal
LA English
CC 4-3 (Toxicology)
AB The disposition of [1-14C]butanal oxime (BOX) was detd. in the rat after oral, i.v. and dermal administration. Oral doses of [14C]BOX (2 and 20 mg/kg) were predominantly excreted in the urine (> 42 %) and converted to 14CO2 (> 30%) and about 10 % of the dose remained in the tissues 72 h post-dosing. Eight and 16% of a 2 and 20 mg/kg dermal dose of BOX, resp., were absorbed, due in part to rapid volatilization from the surface of the skin. Oral doses of BOX were transformed into several polar and/or anionic metabolites that include sulfate conjugates and a significant amt. of thiocyanate. The effect of inhibitors on the metab. of BOX was investigated using 1-aminobenzotriazole (ABT; an inhibitor of diverse cytochrome P450s) and trans-1,2-dichloroethylene (DCE; an inhibitor of CYP2E1). No thiocyanate anion was detected in the urine of rat treated with DCE or ABT. ABT markedly increased the prodn. of 14CO2 and excretion as volatile metabolites. DCE had no effect on 14CO2 excretion, but increased exhalation of radiolabel. ABT also effectively blocked the expression of toxic effects attributable to cyanide in rat given near-LDs of BOX. The data are consistent with two distinct pathways of metab. for BOX, (1) redn. to an imine, hydrolysis and subsequent conversion of butyraldehyde to 14CO2 and (2) CYP3A-catalyzed dehydration of BOX to butyronitrile followed by CYP2E1-catalyzed release of cyanide.
ST butanal oxime disposition metab
IT Adipose tissue
Blood
Feces
Kidney
Liver
Muscle
Organ, animal
Skin
Testis
Urine
(disposition and metab. of butanal oxime following oral, i.v. and dermal administration)
IT 110-69-0, Butanal oxime
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(disposition and metab. of butanal oxime following oral, i.v. and dermal administration)
IT 9035-51-2, **Cytochrome P450**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(disposition and metab. of butanal oxime following oral, i.v. and **dermal** administration)
IT 124-38-9, Carbon dioxide, biological studies 302-04-5, Thiocyanate, biological studies
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(disposition and metab. of butanal oxime following oral, i.v. and dermal administration)

RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE

(1) American Chemical Society; Chemcyclopedia 1996, P46
(2) Boucher, J; Biochemistry 1994, V33, P7811 CAPLUS
(3) de Montellano, O; Biochemical Journal 1981, V195, P761
(4) Demaster, E; Biochemical Pharmacology 1993, V46, P117 CAPLUS
(5) Demaster, E; Journal of Organic Chemistry 1992, V57, P5074 CAPLUS
(6) Forsander, O; Biochemical Pharmacology 1970, V19, P2131 CAPLUS
(7) Gargas, M; Inhalation Toxicology 1990, V2, P295 CAPLUS
(8) Hayes, W; Toxicology and Applied Pharmacology 1967, V11, P327 CAPLUS
(9) Hes, J; Drug Metabolism and Disposition 1974, V2, P345 CAPLUS
(10) Koe, B; Journal of Pharmacology and Experimental Therapeutics 1970, V174, P434 CAPLUS

L19 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS
AN 1991:76751 CAPLUS
DN 114:76751
TI Influence of repeated heavy pyrolysis tarn skin applications on the cytochrome P-450 level and microsomal and cytosol glutathione-S-transferase activity in rat liver. Interrelations between the indexes and toxic effect levels of heavy pyrolysis tar on internal organs
AU Kravchenko, M. N.; Loginov, A. S.; Petrova, L. P.; Ausheva, L. Kh.; Bendikov, E. A.
CS Inst. Ind. Hyg. Occup. Des., Moscow, USSR
SO Byulleten Eksperimental'noi Biologii i Meditsiny (1990), 110(10), 365-7
CODEN: BEBMAE; ISSN: 0365-9615
DT Journal
LA Russian
CC 4-6 (Toxicology)
AB Rats received 20 skin applications of heavy pyrolysis tar, contg. .apprx.30% of polycyclic arom. hydrocarbons. The exposure duration was 4 h/day, 5 days/weak, for 4 weaks. Induction of cytochrome P 450 (P 450) by 79%, induction of microsomal (GTm) and cytosol (GTc) glutathione-S-transferase activity (by 46 and 85%, resp.) and small increase of GSH level (by 9%) were registered after the exposures. Close correlation was obsd. between ratios P 450/GTm and P 450/GTc and toxic effects of heavy pyrolysis tar on rat immune and endocrine systems.
ST pyrolysis tar liver cytochrome GSH transferase
IT Microsome
(glutathione transferase of liver, dermal exposure to heavy pyrolysis tar effect on)
IT Liver, toxic chemical and physical damage
(heavy pyrolysis tar toxicity to, cytochrome P 450 and GSH and glutathione transferase of liver response to)
IT Cytoplasm
(cytosol, glutathione transferase of liver, dermal exposure to heavy pyrolysis tar effect on)
IT Aromatic hydrocarbons, biological studies
RL: BIOL (Biological study)
(polycyclic, of heavy pyrolysis tar, cytochrome P 450 and GSH and glutathione transferase of liver response to dermal exposure to)
IT Petroleum refining residues
(pyrolytic tars, heavy, cytochrome P 450 and GSH and glutathione transferase of liver response to dermal exposure of)
IT 50812-37-8, Glutathione S-transferase
RL: BIOL (Biological study)
(of liver cytosol and microsomes, dermal exposure to heavy pyrolysis tar effect on)
IT 70-18-8, GSH, biological studies 9035-51-2, **Cytochrome P450**, biological studies
RL: BIOL (Biological study)
(of liver, **dermal** exposure to heavy pyrolysis tar effect on)

=>

L7 ANSWER 5 OF 6 USPATFULL

DETD In the **cytochrome P450** pathway, in vivo induction of a donor using phenobarbital upregulates CYPIIB1 and CYPIIB2 isozymatic activity of hepatocytes, or the activity of their porcine homologs, on benzyloxyresorufin (BROD) and pentoxyresorufin (PROD) substrates, respectively. Beta-**naphthoflavone** is specific for upregulation of CYPIA2 and CYPIA1 isozymatic activity, or the activity of their porcine homologs, on methoxyresorufin (MROD) and ethoxyresorufin (EROD) substrates, respectively. Methylcholanthrene upregulates CYPIIB1 isozymatic activity, or its porcine homolog, to PROD; CYPIA2 isozymatic activity, or its porcine homolog, on MROD; and CYPIA1 isozymatic activity, or its porcine homolog, on EROD. Another widely used substrate to assess hepatic enzymatic activity is 7-ethoxycoumarin (7-EC). This substrate is O-deethylated to yield a fluorescent product and is also indicative of oxidative metabolism of the **cytochrome P450** enzymes. The results from these assays suggest that increases in isozymatic function are obtained following in vivo induction. Furthermore, HPLC analysis of the detoxification processes in the liver show that drugs, such as lidocaine and diazepam, which are metabolized in the liver, are cleared at a much greater rate than in the noninduced state. This finding is clinically significant as drug overdoses are a major cause of hepatic failure.

ACCESSION NUMBER: 2002:121845 USPATFULL

TITLE: Vivo induction for enhanced function of isolated hepatocytes

INVENTOR(S): Sullivan, Susan J., Newton, MA, United States
Gregory, Paul G., Shrewsbury, MA, United States
DiMilla, Paul A., Dover, MA, United States

PATENT ASSIGNEE(S): Organogenesis Inc., Canton, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6394812	B1	2002052

L7 ANSWER 6 OF 6 USPATFULL

DETD When the protein is a P450 1A2 or 1A1, the invention provides that a strong inhibitory ligand, e.g. .alpha.-**naphthoflavone** (7,8-benzoflavone), may be added to the membranes before adding the detergents to the fractionated cells. This helps to stabilize the cells and to maintain catalytic activity. Other suitable inhibitory ligands can be determined by comparison to .alpha.-**naphthoflavone** and screened for stabilization activity by the methods set forth in the Examples. Thus, analogs of .alpha.-**naphthoflavone** are included in the invention. When the protein is a human **cytochrome P450** 3A4, it is helpful to add the final step of preincubating the purified protein with glutathione, in order to help stabilize catalytic activity.

ACCESSION NUMBER:	1999:37269 USPATFULL			
TITLE:	Expression and purification of human cytochrome P450			
INVENTOR(S):	Guengerich, F. Peter, Nashville, TN, United States Guo, Zuyu, Nashville, TN, United States Sandhu, Punam, Nashville, TN, United States Gillam, Elizabeth M. J., Queensland, Australia			
PATENT ASSIGNEE(S):	Vanderbilt University, Nashville, TN, United States (U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5886157		19990323	
APPLICATION INFO.:	US 1994-194981		19940210	(8)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Patterson, Jr., Charles L.			
ASSISTANT EXAMINER:	Moore, William W.			
LEGAL REPRESENTATIVE:	Needle & Rosenberg PC			
NUMBER OF CLAIMS:	9			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	62 Drawing Figure(s); 42 Drawing Page(s)			
LINE COUNT:	3483			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 3 OF 6 USPATFULL

DETD The role of different **cytochrome P450** enzymes on the metabolism of 3-methylindole (3MI) was investigated using selective chemical inhibitors. Eight chemical inhibitors of P450 enzymes were screened for their inhibitory specificity towards 3MI metabolism in porcine microsomes: alpha-**naphthoflavone** (CYP1A2), 8-methoxypsoralen (CYP2A6), menthofuran (CYP2A6), sulphaphenazole (CYP2C9), quinidine (CYP2D6), 4-methylpyrazole (CYP2E1), diethyldithiocarbamate (CYP2E1, CYP2A6), and troleandomycin (CYP3A4). The production of the different 3MI metabolites was only affected by the presence of inhibitors of CYP2E1 and CYP2A6 in the microsomal incubations. In a second experiment, a set of porcine microsomes (n=30) was screened for CYP2A6 content by Western blot analysis and also for their 7-hydroxylation activity (CYP2A6 activity). Protein content and enzymatic activity were found to be correlated with 3MI fat content. The results of the present study indicate that measurement of CYP2A6 levels and/or activity is a useful marker for 3MI-induced boar taint.

ACCESSION NUMBER: 2002:230805 USPATFULL

TITLE: Enzymes and metabolites involved in skatole metabolism

INVENTOR(S): Squires, E. James, Guelph, CANADA
Diaz, Gonzalo J., Bogota, CANADA

PATENT ASSIGNEE(S): University of Guelph, Guelph, CANADA (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6448028	B1	20020910	
APPLICATION INFO.:	US 2000-672039		20000929	(9)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 1999-156935P	19990930	(60)

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

L10 ANSWER 15 OF 16 USPATFULL
CLM What is claimed is:
2. The method of claim 1, wherein the flavonoid compound is .alpha.-**naphthoflavone**.

ACCESSION NUMBER:	94:68773 USPATFULL
TITLE:	Use of flavonoids to treat multidrug resistant cancer cells
INVENTOR(S):	Prochaska, Hans J., New York, NY, United States Scotto, Kathleen W., Middle Village, NY, United States
PATENT ASSIGNEE(S):	Sloan-Kettering Institute for Cancer Research, New York, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5336685		19940809	
APPLICATION INFO.:	US 1993-46082		19930412	(8)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Cintins, Marianne M.			
ASSISTANT EXAMINER:	Jarvis, William R. A.			
LEGAL REPRESENTATIVE:	White, John P.			

L10 ANSWER 14 OF 16 USPATFULL

CLM What is claimed is:

1. A method of prophylaxis or treatment of newborn jaundice comprising administering to a subject in need of said prophylaxis or treatment a therapeutically effective amount of a compound which is not habit-forming and which does not cause drowsiness and which does not contain heavy metals or arsenic and which at a concentration of less than 50 .mu.M doubles the quinone reductase specific activity of Hepa 1clc7 cells, said compound being selected from the group consisting of Michael reaction acceptors; diphenols, quinones and compounds which are metabolized to these in Hepa 1clc7 cells; isothiocyanates; fumarates; maleates; 1,2-dithiole-3-thione; beta-**naphthoflavone**; methyl propiolate; and crotonaldehyde.

ACCESSION NUMBER:	96:120914 USPATFULL			
TITLE:	Treatment of newborn jaundice			
INVENTOR(S):	Dannenberg, Andrew J., New York, NY, United States Chowdhury, Jayanta R., New Rochelle, NY, United States			
PATENT ASSIGNEE(S):	Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation) Albert Einstein College of Medicine of Yeshiva University, a Division of Yeshiva University, Bronx, NY, United States (U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5589504		19961231	
APPLICATION INFO.:	US 1994-279899		19940726	(8)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Criares, Theodore J.			
NUMBER OF CLAIMS:	6			

L10 ANSWER 11 OF 16 USPATFULL
CLM What is claimed is:
8. The method of claim 7, wherein the protein is selected from the group consisting of P450 1A2 and 1A1, and the inhibitory ligand is .alpha.-**naphthoflavone** (7,8-benzoflavone).

ACCESSION NUMBER:	1999:37269 USPATFULL
TITLE:	Expression and purification of human cytochrome P450
INVENTOR(S):	Guengerich, F. Peter, Nashville, TN, United States Guo, Zuyu, Nashville, TN, United States Sandhu, Punam, Nashville, TN, United States Gillam, Elizabeth M. J., Queensland, Australia
PATENT ASSIGNEE(S):	Vanderbilt University, Nashville, TN, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5886157		19990323	
APPLICATION INFO.:	US 1994-194981		19940210	(8)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Patterson, Jr., Charles L.			
ASSISTANT EXAMINER:	Moore, William W.			
LEGAL REPRESENTATIVE:	Needle & Rosenberg PC			
NUMBER OF CLAIMS:	9			
EXEMPLARY CLAIM:	1			

uch modes of administration include oral, rectal, **topical**, nasal, interdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis.

CLM What is claimed is:

8. The method of claim 6, wherein the cytochrome P-450 arachidonic acid epoxygenase inducer is selected from the group consisting of 2,3,7,8-tetrachlorodibenzo-p-dioxin, .beta.-**naphthoflavone**, and 3-methylcholanthrene.

ACCESSION NUMBER: 2002:149199 USPATFULL

TITLE: Use of epoxyeicosatrienoic acids in the treatment of cerebrovascular conditions

INVENTOR(S): Liao, James K., Weston, MA, UNITED STATES
Moskowitz, Michael A., Belmont, MA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002077355	A1	20020620
APPLICATION INFO.:	US 2001-870425	A1	20010530 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-207978P	20000530 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: WOLF GREENFIELD & SACKS, PC, FEDERAL RESERVE PLAZA, 600 ATLANTIC AVENUE, BOSTON, MA, 02210-2211
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Page(s)
LINE COUNT: 726
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L25 ANSWER 3 OF 4 USPATFULL

DETD [0017] Inhibition of taxane metabolism may be achieved by administering an effective amount of a CYP3A4 inhibitor and a CYP2C8 inhibitor to a patient receiving taxane treatment. Suitable CYP3A4 inhibitors include ketoconazole, amiodarone, anastrozole, azithromycin, cannabinoids, cimetidine, clarithromycin, clotrimazole, cyclosporine, danazol, delavirdine, dexamethasone, diethyldithiocarbamate, diltiazem, dirithromycin, disulfiram, entacapone (high dose), erythromycin, ethinyl estradoil, fluconazole (weak), fluoxetine, fluvoxamine, gestodene, grapefruit juice, indinavir, isoniazid, itraconazole, metronidazole, mibefradil, miconazole (moderate), nefazodone, nelfinavir, nevirapine, norfloxacin, norfluoxetine, omeprazole (weak), oxiconazole, paroxetine (weak), propoxyphene, quinidine, quinine, quinupristin and dalfopristin, ranitidine, ritonavir, saquinavir, sertindole, sertraline, troglitazone, troleandomycin, valproic acid (weak), verapamil, zafirlukast and zileuton. Suitable CYP2C8 inhibitors include various flavanoids found in foods, such as quercetin, kaempherol, and naringenin; retinoic acid, carbamazepine, tolbutamide, sulfaphenazole, mephenytoin, etc., with quercetin being particularly preferred. The effective amount of the inhibitors to be administered will depend on a number of factors, such as particular inhibitors employed, amount of taxane given, rate of taxane administration (e.g., 1-hour, 3-hour, 24-hour infusion, etc.), route of taxane administration (e.g., i.v. or **oral**), etc. One of ordinary skill could readily determine an effective amount without undue experimentation. In general, for example, a dose of 400-800 mg of ketoconazole and 1-4 grams of quercetin will be effective in conjunction with paclitaxel therapy. The inhibitors are administered to the patient in conjunction with taxane therapy. The inhibitors may be administered prior to the taxane, for example up to about 24 hours before commencing taxane therapy. The inhibitors may be administered concurrently with the taxane, and may even be administered up to about 72 hours after completion of taxane administration, depending on how complete the inhibitation is. The inhibitors can be, but need not be administered to the patient at the same time. The inhibitors may be administered by any suitable route, for example orally, parenterally, intravenously, etc.

DETD [0029] Pretreatment with ketoconazole alone resulted in 59% increase in paclitaxel area under the curve (AUC) (p, 0.05), whereas pretreatment with quercetin one hour prior to paclitaxel had no effect. The combination of ketoconazole and quercetin one hour before paclitaxel resulted in a 146% increase in pharmacokinetic system exposure ($p<0.05$). Because HPLC analysis of quercetin in liver indicated that quercetin levels were high starting 8-12 hours after an **oral** dose, mice were treated on schedules e and f above. Quercetin twelve hours prior to paclitaxel had no effect on system exposure. However, the combination of ketoconazole and quercetin increased paclitaxel AUC by 104% ($p<0.05$). The peak levels of quercetin measured in the livers of mice receiving **oral** supplementation were approximately 15 .mu.M, and occurred between 8 and 12 hours after the dose.

DETD [0030] These data demonstrate that combination pretreatment with **oral** ketoconazole and quercetin can significantly decrease paclitaxel clearance and increase AUC in vivo. This may allow the use of lower doses of paclitaxel to achieve similar system exposure, while decreasing interpatient pharmacokinetic variablity.

CLM What is claimed is:

11. The method of claim 1, wherein the CYP2C8 inhibitor is selected from the group consisting of **quercetin**, kaempherol, naringenin, **retinoic** acid, carbamazepine, tolbutamide, sulfaphenazole, and mephenytoin, and combinations thereof.

17. The composition of claim 14, wherein the CYP2C8 inhibitor is selected from the group consisting of **quercetin**, kaempherol, naringenin, **retinoic** acid, carbamazepine, tolbutamide, sulfaphenazole, and mephenytoin, and combinations thereof.

ACCESSION NUMBER: 2001:205913 USPATFULL
TITLE: Blockade of taxane metabolism
INVENTOR(S): Synold, Timothy W., Monrovia, CA, United States
Doroshow, James H., Arcadia, CA, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001041706	A1	20011115
APPLICATION INFO.:	US 2001-814072	A1	20010322 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-191828P	20000324 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ROTHWELL, FIGG, ERNST & MANBECK, P.C., 555 13TH STREET, N.W., SUITE 701, EAST TOWER, WASHINGTON, DC, 20004
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1

Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

IT 332859-78-6, cytochrome **CYP1A**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(chemoprotective potentials of homoisoflavonoids and chalcones of Dracaena cinnabari in relation to modulations of drug-metabolizing enzymes and antioxidant activity)

RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Ayrton, A; Food Chem Toxicol 1992, V30, P289 CAPLUS
(2) Brown, J; Mutat Res 1980, V75, P243 CAPLUS
(3) Cao, G; Free Rad Biol Med 1997, V22, P749 CAPLUS
(4) Chae, Y; Cancer Lett 1991, V60, P15 CAPLUS
(5) Chaumontet, C; Carcinogenesis 1994, V15, P2325 CAPLUS
(6) Edenharder, R; Mutat Res 1993, V287, P261 CAPLUS
(7) Elangovan, V; Cancer Lett 1994, V87, P107 CAPLUS
(8) Guengerich, F; Carcinogenesis 1991, V11, P2275
(9) Halliwell, B; Food Chem Toxicol 1995, V33, P601 CAPLUS
(10) Hertog, M; J Agric Food Chem 1992, V40, P2379 CAPLUS
(11) Parke, D; Toxicol in Vitro 1990, V4, P680 CAPLUS
(12) Prochaska, H; Meth Nutr Biochem 1994, V5, P360 CAPLUS
(13) Prough, R; Meth Enzymol 1978, V52, P372 CAPLUS
(14) Safe, S; CRC Crit Rev Toxicol 1984, V13, P319 CAPLUS
(15) Shimoi, K; Carcinogenesis 1994, V15, P2669 CAPLUS
(16) Siess, M; Toxicol Appl Pharmacol 1995, V130, P73 CAPLUS
(17) Siess, M; Xenobiotica 1989, V19, P1379 CAPLUS
(18) Smith, P; J Med Chem 1993, V36, P3904
(19) Sogawa, S; J Med Chem 1993, V36, P3904 CAPLUS
(20) Suchy, V; Progress on Terrestrial and Marine Natural Products of Medicinal and Biological Interest 1991, P110
(21) Trela, B; Xenobiotica 1987, V17, P11 CAPLUS
(22) Uchiyama, M; Anal Biochem 1978, V92, P271
(23) Wattenberg, L; Cancer Res 1985, V45, P1 CAPLUS

L12 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 1998:409182 CAPLUS
DN 129:157870
TI Antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of the **cytochrome P450 1A** family

AU Kanazawa, Kazuki; Yamashita, Takatoshi; Ashida, Hitoshi; Danno, Gen-Ichi
CS Department of Biofunctional Chemistry, Faculty of Agriculture, Kobe University, Kobe, 657-8501, Japan

SO Bioscience, Biotechnology, and Biochemistry (1998), 62(5), 970-977
CODEN: BBBIEJ; ISSN: 0916-8451
PB Japan Society for Bioscience, Biotechnology, and Agrochemistry
DT Journal
LA English
CC 4-6 (Toxicology)

AB We found the mechanism in flavonoids that can strongly suppress the mutagenicity of one of the food-derived and carcinogenic heterocyclic amines, 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2). The antimutagenicity was evaluated by IC50 value, the amt. required for 50% inhibition of the mutagenicity of 0.1 nmol Trp-P-2, with Salmonella typhimurium TA98 strain in the presence of S9 mix. The flavones and flavonols were two orders stronger as antimutagens than such antimutagenic phytochems. as chlorophylls and catechins. We had previously found flavonoids to be a desmutagen to neutralize Trp-P-2 before or during attack of DNA, because they had no effect on either the ultimate mutagenic form of Trp-P-2 (N-hydroxy-Trp-P-2) or the mutated cells. The desmutagenicity of the flavonoids did not depend on the hydroxy no. or position that should be assocd. with antioxidative potency, and was also unaffected by the soly. of Trp-P-2 in the assay soln. The inhibitory

effect of the flavonoids on the metabolic activation of Trp-P-2 to N-hydroxy-Trp-P-2 was almost in parallel with the antimutagenic IC50 value, when detd. with a Saccharomyces cerevisiae AH22 cell simultaneously expressing both rat cytochrome P 450 1A1 and yeast reductase. The Ki values of flavones and flavonols for the enzyme were less than 1 .mu.M, while the Km value of Trp-P-2 was 25 .mu.M. The antimutagenicity of the flavones and flavonols was thus concluded to be due to inhibition of the activation process of Trp-P-2 by P 450 1A1 to the ultimate carcinogenic form. They were also able to act as antimutagens toward other indirect mutagens that were activated by P 450 1A1.

ST antimutagenicity flavone flavonol heterocyclic amine cytochrome

IT Structure-activity relationship
(antimutagenic; antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT Mutagens
Mutation inhibitors
Saccharomyces cerevisiae
(antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT Flavones
Flavonoids
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT Amines, biological studies
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(heterocyclic; antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT Flavones
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(hydroxy; antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT 50-32-8, Benzo[a]pyrene, biological studies 70-25-7, Mnng 613-13-8, 2-Aminoanthracene 1162-65-8, Aflatoxin b1 5522-43-0, 1-Nitropyrene 26148-68-5, 2-Amino-9H-pyrido[2,3-b]indole 62450-06-0, Trp-p-1 62450-07-1, Trp-P-2 76180-96-6, Iq 105650-23-5, Phip
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

IT 117-39-5, **Quercetin** 153-18-4, Rutin 154-23-4, Catechin 446-72-0, **Genistein** 480-16-0, Morin 480-19-3, Isorhamnetin 480-40-0, Chrysin 480-41-1, Naringenin 486-66-8, Daidzein 487-26-3, Flavanone 490-46-0, Epicatechin 491-67-8, Baicalein 491-70-3, Luteolin 520-18-3, Kaempferol 520-33-2, Hesperetin 520-36-5, Apigenin 522-12-3, Quercitrin 525-82-6, Flavone 528-48-3, Fisetin 529-44-2, Myricetin 548-83-4, Galangin 552-58-9, Eriodictyol 552-66-9, Daidzin 577-85-5, Flavonol 578-74-5, Apigetrin 855-97-0 863-03-6, Epicatechin gallate 970-74-1, Epigallocatechin 1061-93-4 1064-06-8 1247-97-8 3681-99-0, Puerarin 9035-51-2, Cytochrome P450, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(antimutagenicity of flavones and flavonols to heterocyclic amines by specific and strong inhibition of cytochrome P 450 1A family)

L12 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2002 ACS
AN 1998:404741 CAPLUS
DN 129:94932
TI Inhibition of mouse and human **CYP 1A**-and 2E1-dependent substrate metabolism by the isoflavonoids **genistein** and equol

Generate Collection

L5: Entry 42 of 43 File: USPT Mar 4, 1997

DOCUMENT-IDENTIFIER: US 5607921 A
TITLE: Stabilized cosmetic or dermatological composition containing several precursors of the same active agent in order to maximize its release, and use thereof

CLAIMS:

9. The composition of claim 8, wherein said first precursor is a C.sub.3 to C.sub.6 vitamin or quercetin monosaccharide, and said second precursor is selected from the group consisting of ascorbic acid phosphates, retinol phosphates, tocopherol nicotinates, retinol palmitates, ascorbic acid palmitates, tocopherol acetates, retinol acetates, ascorbic acid acetates, retinol propionates, ascorbic acid propionates, quercetin palmitates, quercetin acetates, quercetin propionates, quercetin ferulates, and mixtures thereof.

23. The composition of ~~claim 22, wherein~~ said skin active agent is selected from the group consisting of vitamin A, vitamin C, vitamin E, lactic acid, quercetin and retinol.

Biochem. J. (1999) 340, 715–722 (Printed in Great Britain)

Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect *CYP1A1* transcription differentially

Henry P. CIOLINO*, Phillip J. DASCHNER† and Grace Chao YEH*

*Cellular Defense and Carcinogenesis Section, Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute–Frederick Cancer Research and Development Center, National Institutes of Health, Building 560, Room 12-05, P. O. Box B, Frederick, MD, USA 21702-1201, U.S.A., and †Intramural Research Support Program, SAIC, National Cancer Institute–Frederick Cancer Research and Development Center, National Institutes of Health, Building 560, Room 12-05, P. O. Box B, Frederick, MD, USA 21702-1201, U.S.A.

Transcriptional activation of the human *CYP1A1* gene (coding for cytochrome P450 1A1) is mediated by the aryl hydrocarbon receptor (AhR). In the present study we have examined the effect of the common dietary polyphenolic compounds quercetin and kaempferol on the transcription of *CYP1A1* and the function of the AhR in MCF-7 human breast cancer cells. Quercetin caused a time- and concentration-dependent increase in the amount of CYP1A1 mRNA and CYP1A1 enzyme activity in MCF-7 cells. The increase in CYP1A1 mRNA caused by quercetin was prevented by the transcription inhibitor actinomycin D. Quercetin also caused an increase in the transcription of a chloramphenicol reporter vector containing the *CYP1A1* promoter. Quercetin failed to induce CYP1A1 enzyme activity in AhR-deficient MCF-7 cells. Gel retardation studies demonstrated that quercetin activated the ability of the AhR to bind to an oligonucleotide containing the xenobiotic-responsive element (XRE) of the *CYP1A1* promoter. These results indicate that quercetin's effect is mediated by the AhR. Kaempferol did not affect *CYP1A1* expression by itself but it inhibited the transcription of *CYP1A1* induced by the prototypical AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), as measured by a decrease in TCDD-induced *CYP1A1* promoter-driven reporter vector activity, and CYP1A1 mRNA in cells. Kaempferol also abolished TCDD-induced XRE binding in a gel-shift assay. Both compounds were able to compete with TCDD for binding to a cytosolic extract of MCF-7 cells. Known ligands of the AhR are, for the most part, man-made compounds such as halogenated and polycyclic aromatic hydrocarbons. These results demonstrate that the dietary flavonols quercetin and kaempferol are natural, dietary ligands of the AhR that exert different effects on *CYP1A1* transcription.

Key words: chemoprevention, flavonoid, MCF-7 cells, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, xenobiotic-responsive element.

INTRODUCTION

Exposure to environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and their halogenated derivatives such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes the induction of the *CYP1A* gene family, which encode cytochromes P450 1A1 and 1A2 [1]. These enzymes catalyse the metabolic activation of PAHs, generating genotoxic metabolites that bind DNA [2] and thus mediate PAH-induced carcinogenesis. Transcriptional activation of *CYP1A1* is regulated by the aryl hydrocarbon receptor (AhR), a cytosolic protein that belongs to the basic helix–loop–helix protein family. The AhR has been detected in several different tissues and cell types [3,4]; it is thought to mediate the broad spectrum of biological responses that PAH or TCDD elicits, including tumorigenesis, teratogenesis, tumour promotion and thymic atrophy [5]. After the binding of PAH or TCDD, the AhR translocates to the nucleus, where it heterodimerizes with a protein partner, the AhR nuclear translocator, forming a transcription factor that binds the xenobiotic-responsive elements (XREs) present in the 5′-promoter of *CYP1A1*, inducing transcription [6]. Several non-PAH compounds have also been shown to be inducers of *CYP1A1* [7–9] but the known ligands of the AhR are mainly man-made compounds. Known natural ligands of the AhR include: indolo[3,2-*b*]carbazole, an acid derivative of a compound found in some vegetables [10–12]; curcumin, a polyphenolic compound found in the spice turmeric [13]; tryptophan metabolites [14]; and bilirubin [15]. Other natural exogenous or endogenous ligands of the AhR have been postulated but not demonstrated.

Flavonoids, a large group of polyphenolic derivatives of benzo-γ-pyrone, are one of the most prevalent class of compounds in edible plants and thus in human diets [16]. Total dietary flavonoid intake has been estimated to be as high as 1 g/day [17] but recent studies have indicated that intake varies widely [18,19]. The most abundant flavonoids are the flavonols quercetin and kaempferol, which exist as a variety of glycosides or in aglycone form. Recent studies have shown that either form of these compounds is absorbed by the human gut [20]. The aglycone forms of quercetin and kaempferol are similar in structure, differing only by one hydroxy group in the B-ring (Figure 1). Quercetin has been extensively studied, particularly with regard to biochemical mechanisms that affect carcinogenesis. In animal models, it has chemopreventive activity against tumorigenesis induced by AhR ligands such as PAHs [21,22]. In cell culture models, it exerts a multiplicity of biochemical effects that are relevant to carcinogenesis, including metal chelation [23], antioxidant properties [24], the inhibition of hepatic enzymes, including CYP1A1, involved in carcinogen activation [25], and the induction of Phase II (conjugating) enzymes [26]. Despite this, there has been to our knowledge no study that has examined the effect of quercetin or kaempferol on the AhR and *CYP1A1* transcription.

We have hypothesized that dietary polyphenolic compounds

Abbreviations used: AhR, aryl hydrocarbon receptor; B[a]P, benzo[a]pyrene; CAT, chloramphenicol acetyltransferase; CYP1A1, cytochrome P450 1A1; DMBA, dimethylbenz[a]anthracene; EMSA, electrophoretic mobility-shift assay; EROD, ethoxyresorufin-O-de-ethylase; GPDH, glyceraldehyde-3-phosphate dehydrogenase; PAH, polycyclic aromatic hydrocarbon; RT–PCR, reverse-transcriptase-mediated PCR; TBE, Tris/borate/EDTA; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; XRE, xenobiotic-responsive element.

To whom correspondence should be addressed (e-mail hciolino@mail.ncifcrf.gov).

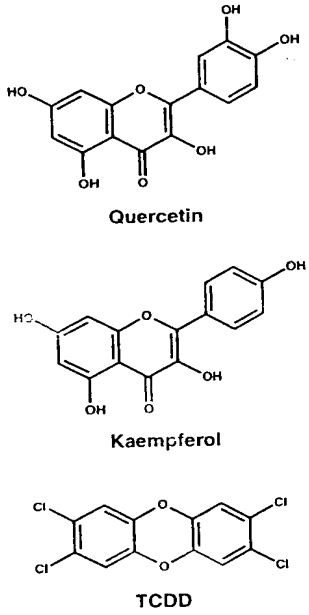


Figure 1 Structures of quercetin, kaempferol and TCDD

such as the flavonoids might be natural ligands of the AhR. This is based on two sets of data: natural ligands of the AhR, indolo[3,2-*b*]carbazole and curcumin, are dietary polyphenolic compounds; and several synthetic derivatives of flavone, the parent structure of flavonoids, are known to interact with the AhR, either as antagonists or as agonists [9,27,28]. To test this hypothesis we examined the effect of the most common and widely distributed flavonoids, quercetin and kaempferol, on *CYP1A1* transcription mediated by the AhR in MCF-7 human breast cancer cells. These cells were chosen as a model system because the function of AhR in these cells has been well characterized [29–31]. We demonstrate that quercetin induces *CYP1A1* transcription by activating the AhR. Although kaempferol does not induce *CYP1A1* transcription, it too interacts with the AhR, and can act as an antagonist of *CYP1A1* transcription induced by TCDD.

MATERIALS AND METHODS

Materials

MCF-7 cells were from the American Type Culture Collection (Rockville, MD, U.S.A.). RPMI 1640, glutamine, fetal bovine serum, trypsin/EDTA, PBS and Tris/borate/EDTA (TBE) buffer were from BioFluids (Rockville, MD, U.S.A.). Quercetin and kaempferol were from Indofine (Somerville, NJ, U.S.A.). Actinomycin D, benzo[*a*]pyrene (B[*a*]P), dimethylbenz[*a*]-anthracene (DMBA), EDTA, dithiothreitol, glycerol, Hepes, polydeoxyinosinic-deoxycytidylic acid, sodium molybdate, ethoxyresorufin, resorufin, Tris/HCl, salmon sperm DNA, DMSO and protease inhibitors were from Sigma (St. Louis, MO, U.S.A.). [^{32}P]dCTP and [^{32}P]dATP were from DuPont NEN (Boston, MA, U.S.A.). [^{3}H]TCDD (specific radioactivity 28.4 Ci/mmol) was from ChemSyn (Lenexa, KS, U.S.A.). Reverse-transcriptase-mediated PCR (RT–PCR) was performed with a kit from Stratagene (La Jolla, CA, U.S.A.). TBE gels, TBE running buffer and high-density sample buffer were from Novex (San Diego, CA, U.S.A.). Primers for glyceraldehyde-3-phosphate dehydrogenase (GPDH) PCR and β-galactosidase-containing reporter vector were from Clontech (Palo Alto, CA, U.S.A.). Bradford protein assay kit was from Bio-Rad (Hercules, CA, U.S.A.). Trizol reagent and LipofectAmine were from Gibco BRL (Gaithersburg, MD, U.S.A.). Chloramphenicol acetyltransferase (CAT) ELISA assay kit was from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Polyclonal antibody against AhR was a gift from Dr. Alan Poland (University of Wisconsin, Madison, WI, U.S.A.).

Cell culture

MCF-7 cells were grown in RPMI 1640 medium supplemented with 2 mM glutamine and 10% (v/v) fetal bovine serum. Cell were subcultured weekly with 0.25% trypsin/0.05% EDTA. All experiments were performed on confluent cultures in growth medium, unless otherwise noted.

RT–PCR

Stock solutions of all chemicals (except where indicated) were made up in DMSO and stored at −20 °C. Control cultures received an amount of DMSO equal to the treated cultures (the final concentration of DMSO was 0.1%). After incubation the cells were washed twice with PBS and total RNA was isolated with Trizol reagent as directed. Semi-quantitative RT–PCR for CYP1A1 mRNA was performed in the presence of 1.5 μCi of [^{32}P]dATP with the primer sequences and conditions of Dohr et al. [29]. cDNA was synthesized from 10 μg of total RNA with the use of a RT–PCR kit as instructed. The optimum cycle number that fell within the exponential range of response for both CYP1A1 (23 cycles) and GPDH (19 cycles) was used. After PCR, 5 μl of high-density sample buffer was added to the samples and they were subjected to electrophoresis on a 10% (w/v) gel in 1 × TBE running buffer. The gel was dried and the results were detected and quantified on a Bio-Rad GS-363 Molecular Imaging System (Hercules, CA, U.S.A.). Graphs of the resulting data were generated by normalizing CYP1A1 to GPDH.

Transient transfections

MCF-7 cells were plated at 60000 cells per well in 24-well plates. After 24 h the cells were transiently co-transfected with 12.0 μg of a CAT reporter vector containing the full-length rat *CYP1A1* promoter [32] and 1.0 μg of a vector containing β-galactosidase with the use of LipofectAmine as directed. The amount of CAT transcription was determined with an ELISA assay as directed. β-Galactosidase activity was determined by the method of Rosenthal [33].

CYP1A1 activity in intact MCF-7 cells

Ethoxyresorufin-*O*-de-ethylase (EROD) activity, which is a specific assay of the bioactivation capacity of CYP1A1, was determined in intact MCF-7 cells grown in 24-well plates as described by Kennedy and Jones [34], with 5 μM ethoxyresorufin in growth medium as a substrate in the presence of 1.5 mM salicylamide to inhibit conjugating enzymes. The assay was performed at 37 °C. The fluorescence of resorufin generated by the conversion of ethoxyresorufin by CYP1A1 was measured every 10 min for 60 min in a CytoFlor II multi-well fluorescence plate reader (PerSeptive Biosystems, Framingham, MA, U.S.A.

with excitation at 530 nm and emission at 590 nm. A standard curve was generated with resorufin.

The AhR-deficient MCF-7 cell line used to determine EROD activity in Figure 5(C) was derived from the parent MCF-7 cells by long-term culture (more than 6 months) in increasing concentrations of the aryl hydrocarbon B[*a*]P. This resulted in the generation of a B[*a*]P-resistant MCF-7 cell line that expresses only approx. 20 % of the AhR of the wild-type cells, as measured at the protein (Western blotting) and mRNA (RT–PCR) levels. EROD activity is not up-regulated in these cells in response to most AhR ligands except high concentrations (10 nM) of the most potent ligand, TCDD. A paper describing these cells is currently in preparation (H. P. Ciolino and G. C. Yeh, unpublished work).

Electrophoretic mobility-shift assay (EMSA)

Confluent cultures of MCF-7 cells were treated as described in the figure legends in growth medium for 3 h. Nuclear protein was isolated and EMSA was performed by the method of Denison et al. [35]. Synthetic oligonucleotides containing the AhR-binding site of the XRE [36] were labelled with [^{32}P]dCTP. The binding reactions were performed for 30 min at room temperature and contained 5 μg of nuclear protein, 1 μg of polydeoxyinosinic-deoxycytidylic acid, 500 ng of salmon sperm DNA and approx. 30000 c.p.m. of labelled probe in a final volume of 20 μl of binding buffer [25 mM Tris/HCl (pH 7.9)/50 mM KCl/1 mM $MgCl_2$/1.5 mM EDTA/0.5 mM dithiothreitol/5 % (v/v) glycerol]. To determine the specificity of binding to the oligonucleotide, a 50-fold excess of unlabelled specific probe, a 50-fold excess of unlabelled non-specific probe of the transcription factor AP-2 or 0.864 μg of anti-AhR polyclonal antibody were incubated with the nuclear extract of quercetin (10 μM)-treated cells on ice for 15 min. DNA–protein complexes were separated under non-denaturing conditions on a 6 % (w/v) polyacrylamide gel with 0.5 × TBE (45 mM Tris borate/45 mM boric acid/2 mM EDTA) as a running buffer. The gels were dried and the DNA–protein complexes were detected and quantified with a Bio-Rad GS-363 Molecular Imaging System.

AhR ligand binding assay

MCF-7 cells were grown to confluence in 175 cm^2 flasks. The cells were washed once in PBS, harvested by treatment with trypsin, and pelleted by centrifugation at 800 *g* for 10 min at 4 °C. The pellet was washed once in cold PBS, repelleted as above and resuspended in cold buffer [25 mM Hepes/1 mM EDTA, 1 mM dithiothreitol/20 mM sodium molybdate/10 % (v/v) glycerol (pH 7.4)] containing protease inhibitors (100 μg/ml PMSF, 300 μg/ml EDTA, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin and 0.7 μg/ml Pepstatin A). The cells were homogenized by 20 strokes with a Dounce glass homogenizer on ice and the homogenate was centrifuged at 100000 *g* for 60 min at 4 °C. The supernatant (cytosol) was removed and protein content was determined by the Bradford method [37]. The cytosol was used immediately or divided into aliquots, stored at −70 °C and used within 24 h. Specific binding to the AHR was measured by sucrose density-gradient centrifugation as described by Raha et al. [38]. Cytosolic protein (1.0 mg) was incubated with 10 nM [^{3}H]TCDD in the presence of DMSO (control), 10 μM unlabelled TCDD (positive control) or 50 μM quercetin or kaempferol in a final volume of 500 μl of the above buffer for 2 h at 4 °C. Samples were applied to 5–30 % (w/v) linear sucrose density gradients in 12 ml Beckman Quick-Seal rotor tubes. The gradients were centrifuged for 2 h at 63000 rev./min (372000 *g*) in a Beckman VTI-65-1 rotor; 25 fractions of seven drops each (approx. 500 μl) were collected from the bottom of the tubes and assayed for radioactivity with Aquasure scintillation fluid. Specific binding to the AhR was also measured by hydroxyapatite absorption chromatography by a modification of the method of Poellinger et al. [39] as described [13].

Statistical analysis

Statistical analyses were performed with STATVIEW Statistical Analysis software (SAS Institute, San Francisco, CA, U.S.A.). Differences between group mean values were determined by a one-factor analysis of variance (ANOVA), followed by Fisher PSLD post-hoc analysis for pairwise comparison of means.

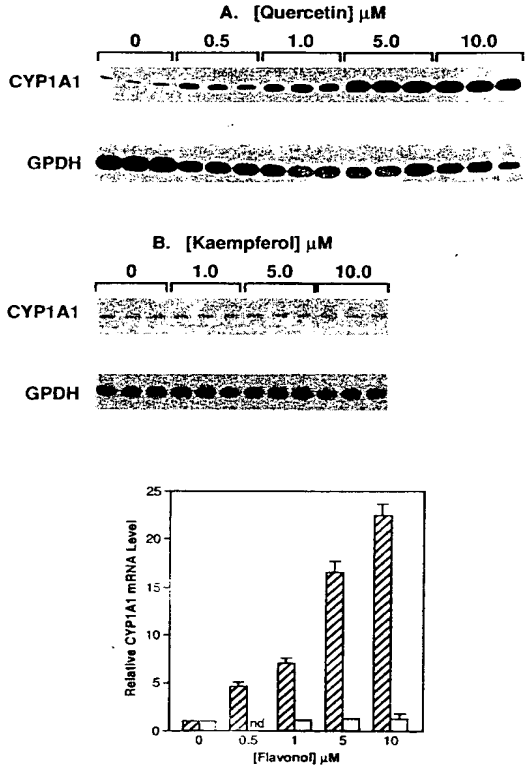


Figure 2 Concentration response of CYP1A1 mRNA to quercetin (A) and kaempferol (B)

MCF-7 cells were treated with the indicated concentration of quercetin (**A**) or kaempferol (**B**) for 24 h RT–PCR for CYP1A1 and GPDH mRNA was performed as described in the Materials and methods section and the results were detected and quantified by phosphorimaging. For the bar chart, the amount of CYP1A1 was normalized to the GPDH level. Hatched bars, quercetin; open bars, kaempferol. Abbreviation: nd, not determined. The level of CYP1A1 mRNA in all quercetin-treated cells was significantly different from control cells ($P < 0.05$).

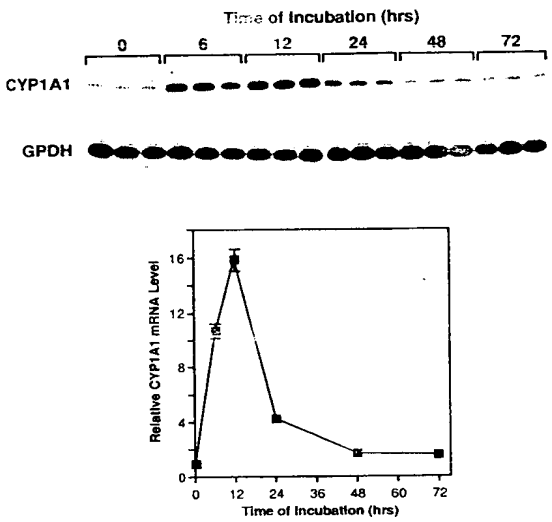


Figure 3 Time course of CYP1A1 mRNA increase caused by quercetin

MCF-7 cells were treated with 0.5 μM quercetin for the durations indicated. CYP1A1 and GPDH mRNA were determined by RT–PCR. For the graph, the amount of CYP1A1 mRNA was normalized to GPDH levels. The level of CYP1A1 mRNA was significantly increased compared with controls after 6, 12 and 24 h of incubation with quercetin ($P < 0.05$).

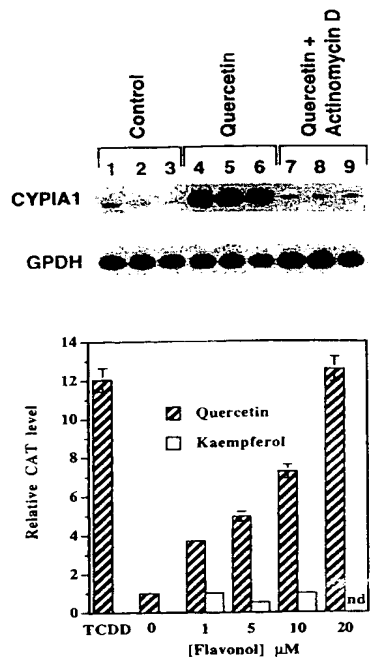


Figure 4 Effect of quercetin or kaempferol on *CYP1A1* transcription

Upper panel: MCF-7 cells were treated for 1 h with ethanol (control) or actinomycin D (5 μg/ml) followed by DMSO (control) or 5 μM quercetin for 6 h; the amount of CYP1A1 and GPDH mRNA was measured by RT–PCR as described. The level of CYP1A1 mRNA in cells treated with quercetin in the presence of actinomycin D was not significantly different from that in control cells. Lower panel: MCF-7 cells were transfected with the aryl hydrocarbon-responsive vector pMC6.3, which contains the *CYP1A1* promoter, and a vector containing β-Gal. Transfected cells were treated with the indicated concentrations of quercetin or kaempferol for 24 h. The amount of CAT transcription was normalized to the amount of β-Gal transcribed. Abbreviation: nd, not determined. CAT transcription in all quercetin-treated samples was significantly increased over that in controls ($P < 0.05$).

RESULTS

Effect of quercetin and kaempferol on the expression of *CYP1A1*

MCF-7 cells were treated with quercetin or kaempferol for 24 h and the amount of CYP1A1 mRNA was measured by semi-quantitative RT–PCR. Quercetin caused a concentration-dependent increase in the amount of CYP1A1 mRNA (Figure 2A), whereas kaempferol had no effect on CYP1A1 mRNA (Figure 2B). Quercetin caused a rapid increase in CYP1A1 transcript that reached a maximum after 12 h of treatment but was still significantly increased after 24 h (Figure 3).

Pretreatment of the cells with the transcription inhibitor actinomycin D abolished the induction of CYP1A1 mRNA caused by quercetin (Figure 4, upper panel).

MCF-7 cells were transfected with a CAT reporter vector containing the full-length *CYP1A1* promoter. Treatment of transfected cells with 1 nM TCDD for 6 h resulted in an increase in CAT transcription of approx. 12-fold over the DMSO control (Figure 4, lower panel). CAT transcription was also increased by treatment with the AhR ligands B[*a*]P, DMBA and 3-methylcholanthrene (results not shown). Quercetin, but not kaempferol, caused a concentration-dependent increase in CAT transcription. This increase reached the approximate level of induction seen in cells treated with 1 nM TCDD (approx. 12-fold over control levels) in cells treated with 20 μM quercetin.

The enzymic activity of CYP1A1 in intact MCF-7 cells treated with quercetin or kaempferol was assayed by measuring EROD activity. Incubation of the cells with quercetin for 48 h caused a concentration-dependent increase in EROD activity over the range of concentrations tested, whereas kaempferol had no effect on EROD activity in the cells (Figure 5A). The quercetin-induced increase in EROD activity was maximal at 48 h but still significantly increased compared with controls after 72 h of incubation (Figure 5B). Wild-type and AhR-deficient MCF-7 cells were incubated with TCDD, B[*a*]P or quercetin for 24 h and the EROD activity was measured after 24 h. Although all three compounds induced EROD activity in varying amounts in wild-type cells, B[*a*]P and quercetin failed to induce EROD activity in AhR-deficient cells, and a high concentration (10 nM) of TCDD induced only approx. 25% of the activity in deficient cells compared with wild-type cells (Figure 5C).

Effect of quercetin on AhR activation

The effect of quercetin on the translocation of the AhR to the nucleus and binding to the XRE of *CYP1A1* was measured by EMSA. Cells were treated with the indicated concentrations of quercetin for 3 h and their nuclear extracts were subjected to EMSA. Extracts from TCDD-treated cells were run as a positive control. Quercetin caused a concentration-dependent increase in the DNA-binding capacity of nuclear AhR, as shown by the

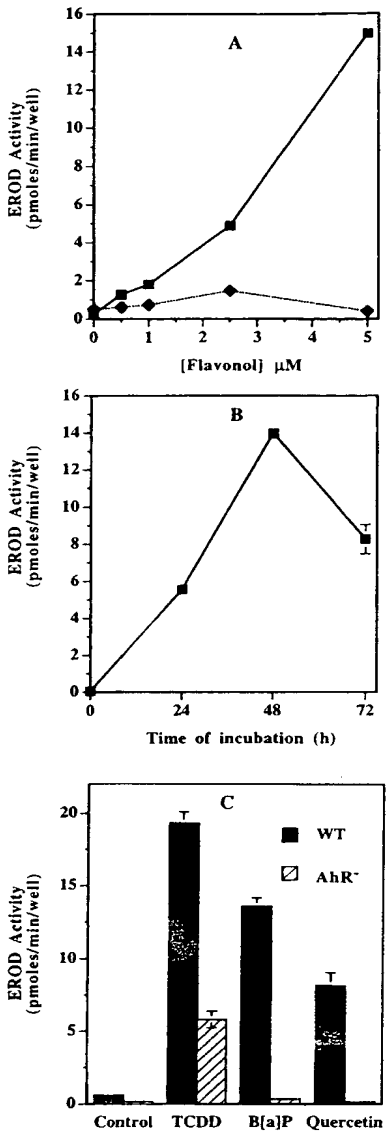


Figure 5 Effect of quercetin or kaempferol on CYP1A1 activity

The activity of CYP1A1 in intact MCF-7 cells was determined by EROD assay. (**A**) Cells were treated with the indicated concentrations of quercetin (■) or kaempferol (◆) for 48 h. (**B**) Cells were treated with 5 μM quercetin for the times indicated. (**C**) Wild-type (WT) and AhR-deficient (AhR⁻) MCF-7 cells were incubated with DMSO (control), 10 nM TCDD, 1 μM B[*a*]P or 5 μM quercetin for 24 h. Each point or bar is the mean ± S.E.M. for four determinations. EROD activity in WT cells (A, B) treated with quercetin was significantly different from that in controls at all concentrations and time points tested ($P < 0.05$). There was no significant difference in EROD activity in AhR⁻ cells treated with B[*a*]P or quercetin compared with that in controls.

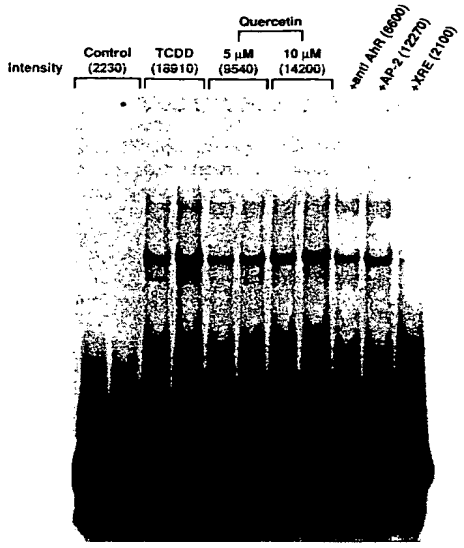


Figure 6 Effect of quercetin on DNA-binding activity of nuclear AhR

Cells were treated with DMSO (control), 10 nM TCDD or the indicated concentrations of quercetin for 3 h. Nuclear extracts were isolated, incubated with labelled XRE sequence and subjected to EMSA. Competition was performed with nuclear extract treated with 10 μM quercetin pretreated with an excess of unlabelled XRE, an oligonucleotide containing the AP-2 sequence, or a polyclonal anti-AhR antibody. The bands were detected and the band intensities quantified by phosphorimaging. The average intensity of each band signal is shown at the top in arbitrary units.

band intensity (arbitrary units) shown at the top of the gel (Figure 6). The specificity of this band shift was examined by pretreating nuclear extract from cells treated with 10 μM quercetin with unlabelled XRE probe, or with a non-specific probe containing the binding site of the transcription factor AP-2. The band shift was abolished in the presence of excess unlabelled XRE but was diminished only slightly in the presence of AP-2 probe. Nuclear extract from quercetin-treated cells was also incubated with a polyclonal antibody against the AhR, which decreased the band intensity by more than 50%. Attempts to super-shift the band with this antibody were unsuccessful.

Effect of quercetin and kaempferol on the binding of ligand to the AhR

The ability of quercetin and kaempferol to compete with the prototypical AhR ligand TCDD for binding to the AhR was measured. Cytosol isolated from MCF-7 cells was incubated with [^{3}H]TCDD in the presence of a 1000-fold excess of unlabelled TCDD (positive control) or a 5000-fold excess of quercetin or kaempferol for 3 h. As shown in Figure 7, unlabelled TCDD inhibited [^{3}H]TCDD binding. Quercetin, and to a smaller extent kaempferol, also inhibited [^{3}H]TCDD binding (see Figure 9). These results were confirmed by using hydroxyapatite chromatography to separate specific from non-specific [^{3}H]TCDD binding (results not shown).

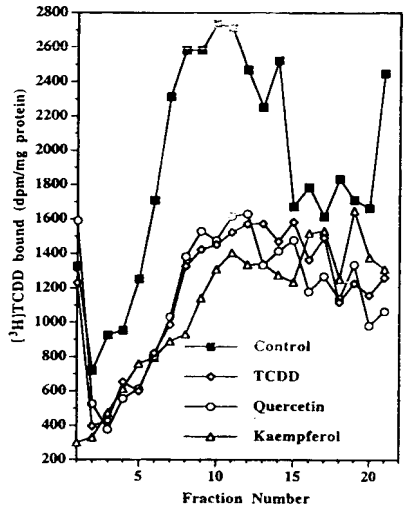


Figure 7 Effect of quercetin or kaempferol on the binding of [^{3}H]TCDD to the AhR

Cytosol isolated from MCF-7 cells was incubated with 10 nM [^{3}H]TCDD in the presence of DMSO (control), a 1000-fold excess of unlabelled TCDD or a 5000-fold excess of quercetin or kaempferol. Ligand-binding activity by the receptor was analysed by sedimentation through 5–30% (w/v) sucrose density gradients; bound [^{3}H]TCDD was measured by liquid-scintillation counting. The figure shows a representative experiment of three.

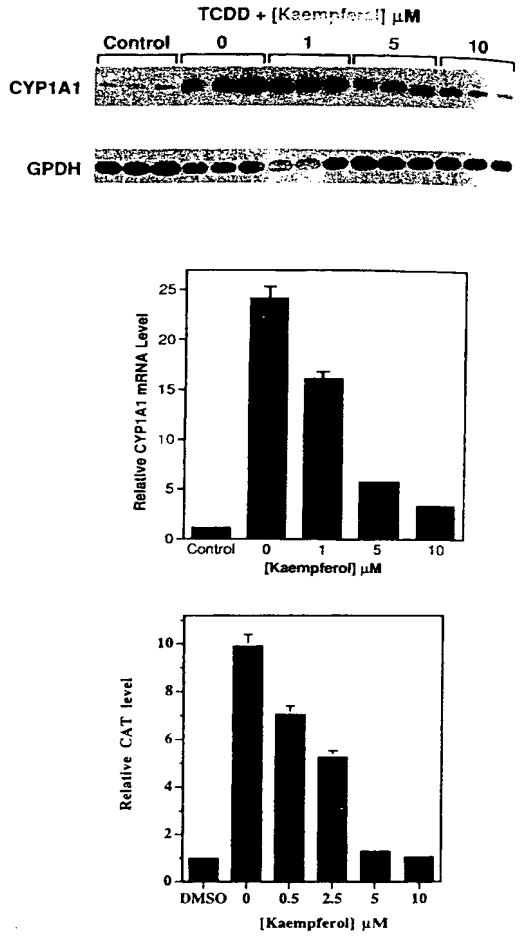


Figure 8 Effect of kaempferol on TCDD-induced CYP1A1 mRNA and transcription

MCF-7 cells were treated with DMSO (control) or 1 nM TCDD in the presence of the indicated concentrations of kaempferol for 6 h. Top and middle panels: CYP1A1 and GPDH mRNA were measured by RT–PCR. Middle panel: the amount of CYP1A1 mRNA was normalized to GPDH mRNA levels. The level of CYP1A1 mRNA was significantly decreased in all samples treated with kaempferol compared to that in cells treated with TCDD alone ($P < 0.05$). Bottom panel: MCF-7 cells were transiently transfected and treated as described above. CAT transcription was normalized to β-Gal transcription. CAT transcription was significantly decreased in all samples treated with kaempferol compared with that in samples treated with TCDD alone ($P < 0.05$).

Effect of kaempferol on the TCDD-induced expression of *CYP1A1*

Although kaempferol did not induce the expression of *CYP1A1*, the results of the ligand binding assay (Figure 7) indicate that it might inhibit the binding of TCDD to the AhR. We therefore tested whether kaempferol could affect the expression of *CYP1A1* induced by TCDD. Treatment of cells with 1 nM TCDD for 6 h caused a 24-fold increase in CYP1A1 transcript compared with that in DMSO-treated cells (Figure 8, top and middle panels). Treatment with TCDD and kaempferol together resulted in an inhibition of TCDD-induced CYP1A1 mRNA in a concentration-dependent manner (Figure 8, top and middle panels). We also examined the effect of kaempferol on *CYP1A1*-promoter-driven CAT transcription. Cells were transfected with the PAH-responsive CAT vector and treated for 6 h with TCDD and kaempferol together. Kaempferol inhibited the TCDD-induced increase in CAT transcription in a concentration-dependent manner (Figure 8, lower panel). The increase in band shift of the XRE caused by TCDD was completely abolished in the presence of kaempferol (Figure 9).

DISCUSSION

Known ligands of the AhR are mainly man-made; natural ligands of the AhR have remained elusive. Two plant-derived dietary compounds, indolo[3,2]carbazole and curcumin, have been shown to be AhR ligands [10–13] and it is therefore likely that the AhR and the pathway that it mediates evolved in response to dietary xenobiotics. If this is so, one would expect at least some of the thousands of chemicals naturally present in the diet to be AhR ligands too. In the present study we have examined the effects of the dietary compounds quercetin and kaempferol on AhR function. These members of the flavonol class of flavonoids are far more widely distributed in the plant kingdom than the compounds mentioned above and are therefore among the most abundant phytochemicals in human diets. Although it has been established that synthetic derivatives of flavone, the parent structure of all flavonoids, might interact with the AhR [9,27,28], the effect of naturally occurring flavonoids

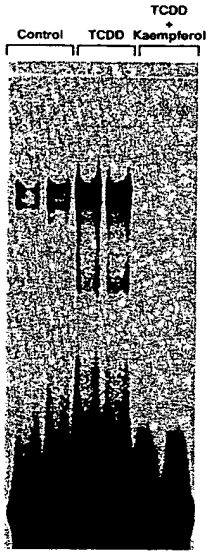


Figure 9 Effect of kaempferol on TCDD-induced DNA-binding activity of nuclear AhR

Cells were treated with DMSO (control), 10 nM TCDD or TCDD and 10 μM kaempferol for 3 h. Nuclear extracts were isolated, incubated with labelled XRE sequence and subjected to EMSA. Bands were detected by phosphorimaging.

on the AhR is largely unexplored. Unfortunately, despite extensive interest in the effects of flavonoids on human health, little is known about the physiologically relevant concentrations of individual flavonoids attainable in human plasma and tissue, but recent experiments have confirmed the absorption of quercetin and kaempferol in humans [19]. Moreover, the concentrations used in this study correspond to plasma levels found in rats fed with a flavonoid-enriched diet [40].

We began by examining the effect of quercetin and kaempferol on the expression of *CYP1A1*. Quercetin induced a concentration-dependent increase in the amount of CYP1A1 mRNA present in MCF-7 cells (Figure 2A). The increase in CYP1A1 mRNA caused by quercetin was rapid but transient, reaching a maximum after 12 h and declining by 24 h (Figure 3). Pretreatment of the cells with the RNA polymerase inhibitor actinomycin D completely blocked the increase in mRNA, indicating that RNA synthesis *de novo* resulting from the transcriptional activation of *CYP1A1* is required for quercetin to exert its effect (Figure 4, upper panel). We examined the effect of quercetin or kaempferol on the transcriptional activation of a CAT reporter vector controlled by the full-length *CYP1A1* promoter. In transient transfection experiments, this vector responded to the prototypical AhR ligand TCDD as well as to other ligands (B[*a*]P, DMBA and 3-methylcholanthrene; results not shown) with an increase in CAT transcription. Quercetin caused a concentration-dependent increase in CAT transcription (Figure 4, lower panel), although it was much less potent an inducer than TCDD.

CYP1A1 encodes the enzyme CYP1A1, the primary carcinogen-activating enzyme in MCF-7 cells under conditions of AhR activation [41]. The enzymic activity of CYP1A1 was measured by EROD assay, the best measurement of its bioactivation capacity. MCF-7 cells also express *CYP1B1* in response to TCDD, but it has been reported that the CYP1B1 enzyme possesses little [42] or no EROD activity [29]. Treatment of MCF-7 cells with quercetin resulted in a concentration- and time-dependent increase in EROD activity in the intact cells (Figures 5A and 5B respectively). EROD activity reached a maximum 48 h after the addition of quercetin; it began to decline after 72 h. As one would expect, the increase in EROD activity follows the increase in CYP1A1 mRNA. Enzyme activity persists much longer than the increase in mRNA, probably reflecting the stability of the enzyme compared with the mRNA. The increases in CYP1A1 mRNA, CYP1A1 enzyme activity and *CYP1A1* promoter-driven transcription indicate that quercetin induces the expression of *CYP1A1*. As shown in Figures 2(B), 4 (lower panel) and 5(A), kaempferol, despite its structural similarity to quercetin, did not affect *CYP1A1* expression.

Because *CYP1A1* transcription is regulated by the AhR, we investigated whether quercetin is a ligand of the receptor. We performed three types of experiment to determine whether quercetin is an AhR ligand. First, we examined the induction of EROD activity in AhR-deficient MCF-7 cells that we have developed and characterized (H. P. Ciolino and G. C. Yeh, unpublished work). These cells express only approx. 20% of the AhR compared with wild-type cells (results not shown). EROD activity in these cells increases only slightly in response to TCDD, the most potent ligand of the AhR, and not at all to other ligands such as B[*a*]P. As shown in Figure 5(C), quercetin failed to induce EROD activity in the AhR-deficient cells, indicating that the AhR is required for quercetin to exert its effect on *CYP1A1* expression. Secondly, we examined the ability of quercetin to transform the cytosolic receptor to its nuclear, DNA-binding, form. As shown in the EMSA in Figure 6, treatment of cells with quercetin resulted in a concentration-dependent increase in the amount of nuclear AhR DNA-binding capacity for an oligonucleotide containing the XRE of the *CYP1A1* promoter. That this band shift was specific for activated AhR is demonstrated by the specific competition of XRE binding of nuclear extracts of quercetin-treated cells with unlabelled XRE probe or anti-AhR antibody. The band also shifted to the same position as that caused by TCDD. Thirdly, we tested the ability of quercetin to compete with TCDD for AhR binding. At a 5000-fold excess, quercetin partly inhibited the binding of [^{3}H]TCDD to the cytosolic AhR (Figure 7). Although the affinity of quercetin for the receptor is therefore low compared with that of TCDD, this result indicates that quercetin interacts directly with the AhR. Taken together, these results demonstrate that quercetin is a ligand of the AhR.

Interestingly, kaempferol also inhibited the binding of TCDD (Figure 7), indicating that it does interact with the AhR. We therefore hypothesized that because kaempferol interacts with the ligand-binding site of the AhR without itself up-regulating transcription, it would antagonize *CYP1A1* transcription induced by TCDD. Treatment of cells with kaempferol and TCDD together resulted in a concentration-dependent decrease in the TCDD-induced increase in both CYP1A1 mRNA (Figure 8, top and middle panels) and CAT transcription (Figure 8, bottom panel). Furthermore, kaempferol completely abolishes the activation of the XRE-binding capacity of the AhR induced by TCDD, as shown in Figure 9. This indicates that kaempferol does in fact interact with the receptor, and therefore is a ligand of the receptor because it functions as an AhR antagonist. It has been shown previously that compounds with weak to moderate binding affinity for the AhR might exhibit partial antagonistic

activity. For example, α-naphthoflavone, a synthetic flavone, inhibits TCDD-induced *CYP1A1* transcription at less than 10 μM, but acts as an agonist at higher concentrations [43]. Similar results were recently obtained with another synthetic flavone, PD98050 [27]. We detected no agonist activity of kaempferol, although concentrations greater than 10 μM were not tested. The mechanism by which kaempferol antagonizes the AhR without any agonistic activity awaits further experimentation.

It is interesting that two compounds so similar in structure as quercetin and kaempferol have such different effects on AhR function. Both compounds fit the profile of AhR ligands: they are polycyclic, planar and hydrophobic. On the basis of computer modelling of known AhR agonists such as TCDD, Kleman et al. [44] determined the molecular structure that allows these compounds to interact tightly with the AhR. AhR ligands were determined to fit a hypothetical rectangle of 6.8 Å × 13.7 Å. This result was confirmed by Lee et al. [45]. Despite the structural similarity of quercetin and kaempferol, it might be that the absence of the extra hydroxy group on the B-ring (Figure 1) prevents kaempferol from achieving an optimal fit into this site, preventing transcriptional activation, while blocking other ligands such as TCDD from binding. Because the induction of *CYP1A1* via the AhR is associated with mutagenic activity of many carcinogens, kaempferol might therefore prove to be an effective chemopreventive agent. In contrast, whether *CYP1A1* induction is harmful or helpful to the organism is a complex question that has not been resolved. One could argue that the induction of *CYP1A1* by quercetin might increase the rate of detoxification of PAHs, because PAH metabolites are better substrates for Phase II enzymes. Therefore quercetin might be chemopreventive, especially if it causes a co-ordinate induction of both *CYP1A1* and the Phase II enzymes, several of which are known to be regulated by the AhR [46].

In this study we have demonstrated that quercetin and kaempferol are natural, dietary ligands of the AhR. In general, most inducers of *CYP1A1* are metabolized by the enzyme that it encodes, TCDD being one prominent exception. If this pathway has evolved in response to such phytochemicals, one could hypothesize that they would be catabolized by CYP1A1. Because quercetin, like B[*a*]P or DMBA (results not shown), induces a transient increase in CYP1A1 mRNA and EROD activity in MCF-7 cells, it might be undergoing catabolic breakdown. Whether this activity is due directly to the activity of CYP1A1 is currently under investigation.

This work was supported in part with Federal funds from the National Cancer Institute, NIH, under contract no. NO1-CO-56000.

REFERENCES

1 Whitlock, Jr., J. P., Chichester, C. H., Bedgood, R. M., Okino, S. T., Ko, H. P., Ma, Q., Dong, L., Li, H. and Clarke-Katzenberg, R. (1997) Drug Metab. Rev. **29**, 1107–1127
2 Peltonen, K. and Dipple, A. (1995) J. Occup. Environ. Med. **37**, 52–58
3 Carver, L. A., Hogenesch, J. B. and Bradfield, C. A. (1994) Nucleic Acids Res. **22**, 3038–3044
4 FitzGerald, C. T., Fernandez-Salguero, P., Gonzalez, F. J., Nebert, D. W. and Puga, A. (1996) Arch. Biochem. Biophys. **333**, 170–178
5 McGregor, D. B., Partensky, C., Wilbourn, J. and Rice, J. M. (1998) Environ. Health Perspect. **106**, 755–760
6 Hankinson, O. (1995) Annu. Rev. Pharmacol. Toxicol. **35**, 307–340
7 Dzeletovic, N., McGuire, J., Daujat, M., Tholander, J., Ema, M., Fujii-Kuriyama, Y., Bergman, J., Madsen, P. and Poellinger, L. (1997) J. Biol. Chem. **272**, 12715–12718
8 Gasiewicz, T. A., Kende, A. S., Rucci, G., Whitney, B. and Willey, J. J. (1996) Biochem. Pharmacol. **52**, 1787–1803
9 Sadar, M. D., Westlind, A., Blomstrand, F. and Andersson, T. B. (1996) Biochem. Biophys. Res. Commun. **229**, 231–237
10 Bradfield, C. A. and Bjeldanes, L. F. (1987) J. Toxicol. Environ. Health **21**, 311–323
11 Bjeldanes, L. F., Kim, J. Y., Grose, K. R., Bartholomew, J. C. and Bradfield, C. A. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 9543–9547
12 Chen, I., Safe, S. and Bjeldanes, L. (1996) Biochem. Pharmacol. **51**, 1069–1076
13 Ciolino, H., Daschner, P., Wang, T. and Yeh, G. (1998) Biochem. Pharmacol. **56**, 197–206
14 Heath-Pagliuso, S., Rogers, W. J., Tullis, K., Seidel, S. D., Cenijn, P. H., Brouwer, A. and Denison, M. S. (1998) Biochemistry **37**, 11508–11515
15 Phelan, D., Winter, G. M., Rogers, W. J., Lam, J. C. and Denison, M. S. (1998) Arch. Biochem. Biophys. **357**, 155–163
16 Hollman, P. C. and Katan, M. B. (1998) Arch. Toxicol. Suppl. **20**, 237–248
17 Kuhnau, J. (1976) World Rev. Nutr. Diet **24**, 117–191
18 Hertog, M. G., Hollman, P. C., Katan, M. B. and Kromhout, D. (1993) Nutr. Cancer **20**, 21–29
19 Hollman, P. C., van Trijp, J. M., Buysman, M. N., van der Gaag, M. S., Mengelers, M. J., de Vries, J. H. and Katan, M. B. (1997) FEBS Lett. **418**, 152–156
20 Hollman, P. C. and Katan, M. B. (1997) Biomed. Pharmacother. **51**, 305–310
21 Mukhtar, H., Das, M., Khan, W. A., Wang, Z. Y., Bik, D. P. and Bickers, D. R. (1988) Cancer Res. **48**, 2361–2365
22 Balasubramanian, S. and Govindasamy, S. (1996) Carcinogenesis **17**, 877–880
23 Brown, J. E., Khodr, H., Hider, R. C. and Rice-Evans, C. A. (1998) Biochem. J. **330**, 1173–1178
24 Cao, G., Sofic, E. and Prior, R. L. (1997) Free Radical Biol. Med. **22**, 749–760
25 Sousa, R. L. and Marletta, M. A. (1985) Arch. Biochem. Biophys. **240**, 345–357
26 Uda, Y., Price, K. R., Williamson, G. and Rhodes, M. J. (1997) Cancer Lett. **120**, 213–216
27 Reiners, Jr., J. J., Lee, J. Y., Clift, R. E., Dudley, D. T. and Myrand, S. P. (1998) Mol. Pharmacol. **53**, 438–445
28 Lu, Y. F., Santostefano, M., Cunningham, B. D., Threadgill, M. D. and Safe, S. (1996) Biochem. Pharmacol. **51**, 1077–1087
29 Dohr, O., Vogel, C. and Abel, J. (1995) Arch. Biochem. Biophys. **321**, 405–413
30 Moore, M., Wang, X., Lu, Y. F., Wormke, M., Craig, A., Gerlach, J. H., Burghardt, R., Barhoumi, R. and Safe, S. (1994) J. Biol. Chem. **269**, 11751–11759
31 Wang, X., Thomsen, J. S., Santostefano, M., Rosengren, R., Safe, S. and Perdew, G. H. (1995) Eur. J. Pharmacol. **293**, 191–205
32 Sogawa, K., Fujisawa-Sehara, A., Yamane, M. and Fujii-Kuriyama, Y. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 8044–8048
33 Rosenthal, N. (1987) Methods Enzymol. **152**, 704–720
34 Kennedy, S. W. and Jones, S. P. (1994) Anal. Biochem. **222**, 217–223
35 Denison, M. S., Fisher, J. M. and Whitlock, Jr., J. P. (1988) J. Biol. Chem. **263**, 17221–17224
36 Chen, Y. H. and Tukey, R. H. (1996) J. Biol. Chem. **271**, 26261–26266
37 Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
38 Raha, A., Reddy, V., Houser, W. and Bresnick, E. J. (1990) Toxicol. Environ. Health **29**, 339–355
39 Poellinger, L., Lund, J., Dahlberg, E. and Gustafsson, J. A. (1985) Anal. Biochem. **144**, 371–384
40 Manach, C., Morand, C., Texier, O., Favier, M., Agullo, G., Demigne, C., Regerat, F. and Remsy, C. (1995) J. Nutr. **125**, 1911–1922
41 Christou, M., Savas, U., Spink, D., Gierthy, J. and Jefcoate, C. (1994) Carcinogenesis **15**, 725–732
42 Shimada, T., Gillam, E. M., Sutter, T. R., Strickland, P. T., Guengerich, F. P. and Yamazaki, H. (1997) Drug Metab. Dispos. **25**, 617–622
43 Wilhelmsson, A., Whitelaw, M. L., Gustafsson, J. A. and Poellinger, L. (1994) J. Biol. Chem. **269**, 19028–19033
44 Kleman, M. I., Overvik, E., Mason, G. G. and Gustafsson, J. A. (1992) Carcinogenesis **13**, 1619–1624
45 Lee, I. J., Jeong, K. S., Roberts, B. J., Kallarakal, A. T., Fernandez-Salguero, P., Gonzalez, F. J. and Song, B. J. (1996) Mol. Pharmacol. **49**, 980–988
46 Emi, Y., Ikushiro, S. and Iyanagi, T. (1996) J. Biol. Chem. **271**, 3952–3958

Received 4 January 1999/22 March 1999; accepted 14 April 1999

Kuerbitz, S.J., Plunkett, B.S., Walsh, W.V., Kastan, M.B., 1992. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA 89, 7491–7495.

Lane, D.P., 1992. p53, guardian of the genome. Nature 358, 15–16.

Lee, S., Elenbaas, B., Levine, A., Griffith, J., 1995. p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell 81, 1013–1020.

Li, F.P., Fraumeni, J.F. Jr., Mulvihill, J.J., Blattner, W.A., Dreyful, M.G., Tucker, M.A., Miller, R.W., 1988. A cancer family syndrome in twenty-four kindreds. Cancer Res. 48, 5358–5362.

Malkin, D., 1994. p53 and the Li–Fraumeni syndrome. Biochim. Biophys. Acta 1198, 197–213.

Mehta, C.R., Patel, N.R., 1983. A network algorithm for performing Fisher's exact test in r×c contingency tables. J. Am. Stat. Assoc. 78, 427–434.

Nigro, J.M., Baker, S.J., Preisinger, A.C., Jessup, J.M., Hostetter, R., Cleary, K., Bigner, S.H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F.S., Weston, A., Modali, R., Harris, C.C., Vogelstein, B., 1989. Mutations in the p53 gene occur in diverse human tumour types. Nature 342, 705–708.

Nishino, H., Herath, J.F., Jenkins, R.B., Sommer, S.S., 1995a. Fluorescence in situ hybridization for rapid differentiation of zygosity in transgenic mice. BioTechniques 19, 587–592.

Nishino, H., Knöll, A., Buettner, V.L., Frisk, C.S., Maruta, Y., Haavik, J., Sommer, S.S., 1995b. p53 wild-type and p53 nullizygous Big Blue® transgenic mice have similar frequencies and patterns of observed mutation in liver, spleen and brain. Oncogene 11, 263–270.

Nishino, H., Buettner, V.L., Haavik, J., Schaid, D.J., Sommer, S.S., 1996a. Spontaneous mutation in Big Blue® transgenic mice: Analysis of age, gender, and tissue type. Environ. Mol. Mutagen. 28, 299–312.

Nishino, H., Buettner, V.L., Sommer, S.S., 1996b. Towards validation of the Big Blue® transgenic mouse mutagenesis assay: The mutational spectrum of ex vivo pinpoint mutant plaques. Mutation Res. 372, 97–105.

Provost, G.S., Kretz, P.L., Hamner, R.T., Matthews, C.D., Rogers, B.J., Lundberg, K.S., Dycaico, M.J., Short, J.M., 1993. Transgenic systems for in vivo mutation analysis. Mutation Res. 288, 133–149.

Sands, A.T., Suraokar, M.B., Sanchez, A., Marth, J.E., Donehower, L.A., Bradley, A., 1995. p53 deficiency does not affect the accumulation of point mutations in a transgene target. Proc. Natl. Acad. Sci. USA 92, 8517–8521.

Skopek, T.R., 1995. Of mice and mutants: Target size and sensitivity. Mutation Res. 331, 225–228.

Skopek, T.R., Kort, K.L., Marino, D.R., 1995. Relative sensitivity of the endogenous *hprt* gene and *lacI* transgene in ENU-treated Big Blue® B6C3F1 mice. Environ. Mol. Mutagen. 26, 9–15.

Skopek, T.R., Kort, K.L., Marino, D.R., Mittal, L.V., Umbenhauer, D.R., Laws, G.M., Adams, S.P., 1996. Mutagenic response of the endogenous *hprt* gene and *lacI* transgene in benzo[*a*]pyrene-treated Big Blue® B6C3F1 mice. Environ. Mol. Mutagen. 28, 376–384.

Smith, M.L., Chen, I., Zhan, Q., O'Connor, P.M., Fornace, A.J. Jr., 1995. Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage. Oncogene 10, 1053–1059.

Sommer, S.S., Ketterling, R.P., 1994. How precisely can data from transgenic mouse mutation-detection systems be extrapolated to humans?: Lessons from the human factor IX gene. Mutation Res. 307, 517–531.

Stewart, N., Hicks, G.G., Paraskevas, F., Mowat, M., 1995. Evidence for a second cell cycle block at G2/M by p53. Oncogene 10, 109–115.

Stoflet, E.S., Koeberl, D.D., Sarkar, G., Sommer, S.S., 1988. Genomic amplification with transcript sequencing. Science 239, 491–494.

Walker, V.E., Gorelick, N.J., Andrews, J.L., Craft, T.R., DeBoer, J.G., Glickman, B.W., Skopek, T.R., 1996. Frequency and spectrum of ethylnitrosourea-induced mutation at the *hprt* and *lacI* loci in splenic lymphocytes of exposed *lacI* transgenic mice. Cancer Res. 56, 4654–4661.

Wang, X.W., Yeh, H., Schaeffer, L., Roy, R., Moncollin, V., Egly, J., Wang, Z., Friedberg, E.C., Evans, M.K., Taffe, B.G., Bohr, V.A., Weeda, G., Hoeijmakers, J.H.J., Forrester, K., Harris, C.C., 1995. p53 modulation of TFIIH-associated nucleotide excision repair activity. Nature Genet. 10, 188–195.

ELSEVIER

Mutation Research 379 (1997) 21–32

MR
Fundamental and Molecular Mechanisms of Mutagenesis

The inhibition by flavonoids of 2-amino-3-methylimidazo[4,5-*f*]quinoline metabolic activation to a mutagen: a structure–activity relationship study

R. Edenharder [a,*], R. Rauscher [a], K.L. Platt [b]

[a] *Department of Hygiene and Environmental Medicine, University of Mainz, Langenbeckstraße 1, D-55131 Mainz, Germany*
[b] *Institute of Toxicology, University of Mainz, Langenbeckstraße 1, D-55131 Mainz, Germany*

Received 11 September 1996; revised 2 January 1997; accepted 12 March 1997

Abstract

The mutagenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) in *Salmonella typhimurium* TA98 is inhibited by flavonoids with distinct structure–antimutagenicity relationships (Edenharder, R., I. von Petersdorff I. and R. Rauscher (1993) Antimutagenic effects of flavonoids, chalcones and structurally related compounds on the activity of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and other heterocyclic amine mutagens from cooked food, Mutation Res., 287, 261–274). With respect to the mechanism(s) of antimutagenicity, the following results were obtained here. (1) 7-Methoxy- and 7-ethoxyresorufin-*O*-dealkylase activities in rat liver microsomes, linked to cytochrome P-450-dependent 1A1 and 1A2 monooxygenases catalyzing oxidation of IQ to *N*-hydroxy-IQ (*N*-OH-IQ), were effectively inhibited by 16 flavonoids (IC_{50}: 0.4–9.8 μM). Flavones and flavonols are in general more potent enzyme inhibitors than flavanones, isoflavones, and chalcones. Among flavones the presence of hydroxyl or methoxyl groups resulted in minor changes only. However, among flavonols and flavanones the parent compounds exerted the strongest inhibitory effects, which decreased in dependence on number and position of hydroxyl functions. Contrary to the results obtained in the Salmonella assay in the tests with alkoxyresorufins no extraordinary counteracting effects of isoflavones, of hydroxyl groups at carbons 6 or 2′ or of the elimination of ring B (benzylideneacetone) were detected. (2) No effects of flavonoids on NADPH-dependent cytochrome P-450 reductase activity could be detected. (3) The effects of 30 flavonoids on mutagenicity induced by *N*-OH-IQ in *S. typhimurium* TA98NR were again structure dependent. The most striking feature was the, in principle, reverse structure–antimutagenicity pattern as compared to IQ: non-polar compounds were inactive and a 50% inhibition was achieved only by some flavones and flavonols (IC_{50}: 15.0–148 nmol/ml top agar). Within the flavone and flavonol subgroups inhibitory effects increased in dependence on number and position of hydroxyl functions. Isoflavones and flavanones, however, as well as glycosides, were inactive. Hydroxyl groups at carbons 7, 3′, 4′, and 5′ generated antimutagenic compounds, a hydroxyl function at C5 was ineffective, but hydroxyls at C3 and 6 as well as methoxyl groups at C3′ (isorhamnetin) or 4′ (diosmetin)

Abbreviations: DMSO, dimethylsulfoxide; EROD, 7-ethoxyresorufin-*O*-deethylase; HCAs, heterocyclic amines; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MROD, 7-methoxyresorufin-*O*-demethylase; NADPH, nicotinamide-adenine-dinucleotidphosphate (reduced); *N*-OH-IQ, *N*-hydroxy-2-amino-3-methylimidazo[4,5-*f*]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; S9, supernatant of centrifugation at 9000 × *g*; S105, supernatant of centrifugation at 105 000 × *g*

* Corresponding author.

PII S0027-5107(97)00085-7

generated comutagenic compounds. 4. Cytosolic activation of IQ to mutagenic metabolites as determined by experiments with the hepatic S105 fraction comprises about 10% of the mutagenicity after activation by the combined microsomal and cytosolic fractions (S9). The pattern of inhibition as produced by 20 flavonoids was closely similar to that observed with the S9 fraction. 5. In various experiments designed for modulation of the mutagenic response, it could be shown that further mechanisms of flavonoid interaction with the overall mutagenic process may exist, such as interactions with biological membranes (luteolin, fisetin) and effects on fixation and expression of DNA damage (flavone, fisetin). © 1997 Elsevier Science B.V.

Keywords: Cooked food mutagen; Salmonella/reversion assay; Cytochrome P-450; Structure–activity relationship

1. Introduction

When meat and fish, foods rich in protein, are heated under normal household conditions a series of heterocyclic amines (HCAs) are generated. Thus persons consuming a mixed-western diet are exposed to these HCAs for nearly an entire lifetime [2,3]. Five HCAs, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) are considered to be the principal HCAs consumed with the US diet [4]. Although all these HCAs are mutagenic in the Salmonella/reversion assay and carcinogenic in laboratory animals, IQ possesses the highest mutagenic and carcinogenic potency and therefore warrants special attention [4–6]. HCAs were regarded as potential human carcinogens [7], but the real health hazards are still unknown. According to recent calculations [4], the HCAs being typical tumor initiators [5,8] might represent a lower risk as human carcinogens than originally anticipated from the animal experiments. However, promotional effects which cannot be calculated so far could play a more decisive role in determining the carcinogenicity of these compounds.

On the other hand, there is now overwhelming evidence from epidemiological studies that a high consumption of fruit and vegetables is consistently associated with a low incidence of many types of cancer, especially epithelial cancers of the alimentary and respiratory tracts [9–11]. Knowledge about phytochemicals responsible for anticarcinogenic and antimutagenic properties of fruits and vegetables is limited (see recent reviews [12,13]). Among compounds of known structure, flavonoids deserve special attention because they are present in practically all dietary plants, fruits, and roots, are consumed daily in considerable amounts, at least 23 mg according to Hertog et al. [14], and are heat stable. Additionally, flavonoids are non-toxic, exert a wide variety of health-protective physiological and biological properties, including antioxidative effects, scavenging of free radicals, modulation of enzymatic activities, antimutagenic, anticarcinogenic, and anticlastogenic properties [15,16]. In an earlier study, we had investigated 64 flavonoids for their antimutagenic potencies in the Salmonella/reversion assay with respect to IQ and, in part, other HCAs and had detected distinct structure–antimutagenicity relationships [1]. In this communication, we present further results of more detailed investigations performed to elucidate the mechanisms of antimutagenicity of flavonoids with respect to IQ-induced mutagenesis. These include structure–activity relationship studies of flavonoids with respect to inhibition of cytochrome P-450 dependent monooxygenases 1A1 and 1A2, responsible for microsomal hydroxylation of IQ to *N*-OH-IQ, inhibition of bacterial esterification reactions of *N*-OH-IQ, necessary for the formation of ultimate mutagens, and inhibition of the cytosolic activation of IQ to bacterial mutagens as well as investigations on other possibilities of flavonoid interactions, such as effects on biological membranes and on DNA repair [17].

2. Materials and methods

2.1. Chemicals

The mutagens IQ and *N*-OH-IQ were purchased from Toronto Research Chemicals Inc., Downsview, Canada, and from Midwest Research Institute, Kansas City, MO, respectively. Unless otherwise indicated flavonoids as well as benzylideneacetophenone (chalcone) were obtained from Roth, Karlsruhe. Biochanin A, flavanone, benzylideneacetone and resorufin natrium were supplied from Aldrich-Chemie, Steinheim. Quercetin and flavone were purchased from Fluka-Deutschland, Neu-Ulm, while genistein was from ICN-Biomedicals, Meckenheim. Ampicillin and β-naphthoflavone were obtained from Serva, Heidelberg. Aroclor 1254 was from Bayer, Leverkusen. Glucose-6-phosphate and 7-ethoxyresorufin were purchased from Boehringer, Mannheim. Cytochrome *c* (from horse heart) was supplied by Sigma, Deisenhofen. 7-Methoxyresorufin was prepared as described [18]. All other chemicals were obtained from Merck, Darmstadt, Germany. All flavonoids and other compounds tested in mechanistic investigations of antimutagenicity were of the highest purity grade available.

2.2. Mammalian metabolic activation system (S9 mix), preparation of microsomes and of S105 fraction for cytosolic activation

Male Sprague–Dawley rats (200–270 g), purchased from Interfauna, Süddeutsche Versuch-stierfarm, Tuttlingen, were treated for enzyme induction either with Aroclor 1254 (500 mg/kg) or with β-naphthoflavone (80 mg/kg) [19]. According to the latter recommendation, 3 doses of 80 mg/kg β-naphthoflavone, dissolved in corn oil, were given by oral gavage on days 3, 2 and 1 before the rats were killed. S9 prepared in this way was generally used for all investigations in this study unless otherwise indicated. The S9 content in the S9 mix was reduced to 8.3%, half the concentration recommended by Maron and Ames [20]. However, when S105 was used, the concentration was 16.6%. Further procedures were as described [20]. Microsomes were isolated from the S9 fraction of Aroclor 1254-treated rats by further centrifugation at $100\,000 \times g$ for 90 min. The microsomal pellet was resuspended in isotonic KCl, centrifuged again as described and finally resuspended in phosphate buffered isotonic KCl, pH 7.4 and stored in small aliquots at −80°C until use. The supernatant was twice more centrifuged at $105\,000 \times g$ for 90 min and applied for investigations concerning the cytosolic activation of IQ. The protein content was measured by the method of Lowry et al. [21] using bovine serum albumin as a standard.

2.3. Enzyme assays

The activity of NADPH-cytochrome P-450 reductase was spectrophotometrically measured by the reduction of cytochrome c (50 μM) at 550 nm [22] in the presence of 1 mM KCN to block non-microsomal enzyme activity due to possible mitochondrial contamination. Under control conditions enzymatic activity was 323 nmol/min/mg protein.

The cytochrome P-450-dependent 7-alkoxyresorufin-*O*-dealkylase activities were determined fluorimetrically (excitation 522 nm, emission 586 nm, slit width 5 nm) [23] measuring resorufin liberated from 7-ethoxy- or 7-methoxyresorufin (2 μM, each).

2.4. Toxicity testing

Bacterial cytotoxicity of flavonoids and other compounds used in this study for investigations on antimutagenicity had already been determined previously [1] according to the methods of Waleh et al. [24]. None of these compounds was toxic up to a concentration of 133 nmol/ml top agar.

2.5. Mutagenicity testing

Direct mutagenicity of flavonoids in Salmonella depends on a multiplicity of environmental factors including oxygen concentration and on various cellular factors [13]. Flavonoids were checked for direct mutagenicity in *S. typhimurium* TA98NR in concentrations of 200 and 500 nmol/plate under the conditions described below (cf. Section 2.6). None of the flavonoids tested was mutagenic except quercetin which was then investigated in doses of 50, 100, 250, 500, 750 and 1000 nmol/plate. The following numbers of his^+ revertants/plate were obtained: 250, 248, 320, 360, 320 and 390, (spontaneous revertants subtracted, values are means of triplicate plates). These results agree with data published by Nagao et al. [25] with the exception of isorhamnetin. However, non-mutagenicity of isorhamnetin was also reported by Czeczot et al. [26].

2.6. Antimutagenicity testing

Antimutagenicity testing of flavonoids and structurally related compounds against IQ was performed as previously described [1] following with minor modifications the procedures reported by Maron and Ames [20]. When *N*-OH-IQ was used instead of IQ, the tests were executed as described [18]. The actual test assay was as follows: 500 μl isotonic KCl (0.15 M in 0.01 M sodium phosphate buffer, pH 7.4), 25 ng IQ, dissolved in 50 μl DMSO, 100 μl DMSO solution of the test compound, 500 μl S9 (or S105) mix, 100 μl bacterial suspension and 2.5 ml top agar, total volume 3.75 ml. When the directly acting mutagen *N*-OH-IQ was used, 500 μl S9 mix was replaced by 500 μl isotonic KCl. In these tests, 1.5 ng *N*-OH-IQ (stored at −80°C until use), dissolved in 40 μl 0.05 M KH_2PO_4 buffer, pH 4.5, were applied and the strain *S. typhimurium* TA98 was replaced by strain TA98NR (deficient for nitroreductase activities). Actual doses and his^+-revertants numbers were as given follows. *S. typhimurium* TA98: IQ, 25 ng; S9, 2706 ± 366 revertants/plate ($n = 26$); S105, 659 ± 47 revertants/plate ($n = 12$). *S. typhimurium* TA98NR: *N*-OH-IQ, 1.5 ng; 1026 ± 58 revertants/plate ($n = 22$). Dose–response curves were constructed from measurements with 8–12 different doses of flavonoids, performed in duplicate. All data given in the tables are means from two independent series.

2.7. Methodological variations of the Salmonella/reversion assay

Pre- and post-treatment procedures were as described by de Flora et al. [17]. In pretreatment experiments, bacterial cells were incubated with the flavonoid in doses of 100, 250 or 500 nmol/test, dissolved in 200 μl DMSO, for 4 h, isolated by centrifugation, and washed three times, incubated with either 25 ng/test IQ in the presence of S9 (*S. typhimurium* TA98) or 1.5 ng/test *N*-OH-IQ for 20 min, centrifuged and washed again, resuspended and plated after the addition of top agar. All measurements were performed three times. Under these conditions, 25 ng IQ induced 2150 ± 180 and 1.5 ng *N*-OH-IQ 1110 ± 74 revertants/plate.

The post-treatment procedure was executed in three variations.

Variation A: 500 μl isotonic KCl (pH 6.5), 100 μl bacterial suspension, and 2 ng *N*-OH-IQ dissolved in 55 μl 0.05 M KH_2PO_4 buffer were incubated by gentle shaking at 37°C for 20 min. Then the bacteria were washed three times in 5 ml isotonic KCl (pH 6.5), resuspended in 500 μl of this buffered KCl, 100 μl DMSO solution of flavone or fisetin in doses of 100, 200, or 400 nmol/test and 2.5 ml top agar were added and plated.

Variation B: assay as under A with the following difference. After the first washing bacteria were resuspended in 5 ml of fresh nutrient broth, 100 μl DMSO solution of flavone or fisetin in the respective concentration was added and the assay was incubated by gentle shaking at 37°C for 30 min. After three additional washings, the pellet was resuspended in 500 μl isotonic KCl (pH 6.5), 2.5 ml top agar were added and plated.

Variation C: assay as under A with the following difference. After the first washing bacteria were resuspended in 5 ml of fresh nutrient broth and incubated by gentle shaking at 37°C for 30 min. After three washings of the bacteria, the pellet was resuspended in 500 μl isotonic KCl (pH 6.5), 100 μl DMSO solution of flavone or fisetin in doses of 100, 200, or 400 nmol/test and 2.5 ml top agar were added and plated.

2.8. Statistical analysis

Comparison of data was carried out according to Student's *t*-test and the Wilcoxon-test.

3. Results

Activities of the cytochrome P-450-dependent monooxygenases 1A1 and 1A2 were followed by fluorimetric measurement of 7-ethoxyresorufin-*O*-deethylase (EROD) and 7-methoxyresorufin-*O*-demethylase (MROD), respectively, in the presence of flavonoids (Fig. 1) for a total of 16 compounds (Table 1). As can be seen from this table, the concentrations which caused a 50% reduction of rat liver MROD activities (IC_{50} values) were independent of the inducer, Aroclor 1254 or β-naphthoflavone. Again, MROD and EROD showed similar responses to flavonoids except for flavanone and 2′-hydroxyflavanone. However, distinct differences

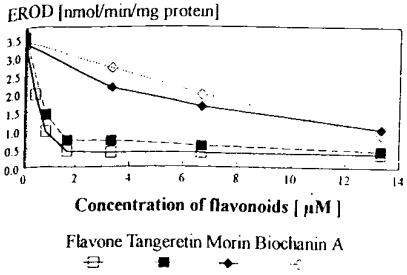


Fig. 1. Inhibition of 7-ethoxyresorufin-O-deethylase (EROD) activity in liver microsomes of Aroclor 1254-treated rats by flavonoids. Each point represents the average of three measurements. All series were performed in duplicate. EROD activity was determined fluorimetrically measuring the amount of resorufin liberated.

between IC_{50} values obtained with the resorufin assays and the Salmonella/reversion assay were evident for 6-hydroxyflavone, apigenin, morin, 2′-hydroxyflavanone, genistein, biochanin A and benzylideneacetone. Among flavones and flavonols, the parent compounds themselves as well as an other relatively non-polar flavone, tangeretin, were the most potent inhibitors of MROD and EROD activities. Additional hydroxyl groups did not change much except in the case of fisetin, morin and possibly 6-hydroxyflavone with hydroxyl functions in positions 3′, 2′ or 6, respectively, which seem to cause a decrease in inhibitory potency. The interfering effects of a 3′- or 2′-hydroxyl function were again observed in the flavanone series with hesperetin and 2′-hydroxyflavanone. The parent compound flavanone, however, inhibited MROD, but not EROD, activities to a similar degree as flavone. The isoflavonoids genistein and biochanin A, as well as chalcone (benzylideneacetophenone) and benzylideneacetone, were less potent inhibitors of MROD and EROD activities.

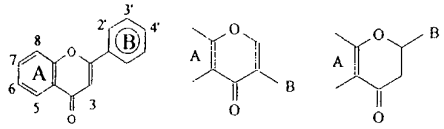
Next, we investigated the influence of the flavonoids compiled in Table 1 (except the isoflavones, but in addition chrysin and naringenin) in concentrations up to 40 μM on the activity of NADPH-dependent cytochrome P-450 reductase which is an indispensable part of a functional monooxygenase system. There were no significant effects detected with any compound except with chalcone which weakly (31% reduction) inhibited this enzyme (data not shown).

When the effects of a total of 30 flavonoids on the mutagenicity of *N*-OH-IQ in *S. typhimurium* TA98NR were investigated again distinct structure–activity relationships were observed. As can be seen from Table 2, non-polar and relatively non-polar flavonoids, such as flavone, flavanone, chalcone, 6-methoxyflavone, and tangeretin, were inactive or even exerted some comutagenicity (benzylideneacetone). Again, in agreement with expectation, two flavonoid glycosides were inactive. In the flavone and flavonol series, antimutagenic potency depended on the number and position of polar hydroxyl functions. Among monohydroxylated flavones available, only a OH-group at carbon 7 caused antimutagenicity, while a hydroxyl function at C5 rendered the flavone inactive or was even counteracting when arranged in positions 6 and 3. Compounds of increasing antimutagenic potency were generated when three or more hydroxyl functions were present, especially when positions 3′ and 4′ were concomitantly substituted: luteolin, fisetin and myricetin (IC_{50}: 15–33.6 nmol/ml top agar) were among the most potent flavonoids, while quercetin and robinetin were less effective. This may reflect the interfering effect of a 3-hydroxyl group which is also detectable when kaempferol and apigenin are compared. On the other hand, methylation of a 3′- or 4′-OH-group in diosmetin and isorhamnetin eliminated antimutagenicity and generated comutagenicity. The real situation may still be more complex since the dose–response curves obtained with these two flavonoids passed a maximum of activity. Methylation of the 4′-OH function in kaempferoltrimethylether also produced a comutagenic compound. In the isoflavonoid series, only weak inhibitory effects were observed with genistein and biochanin A, while some flavanones were either inactive or exerted weak inhibiting or enhancing effects on mutagenicity of *N*-OH-IQ in *S. typhimurium* TA98NR.

In order to further elucidate the mechanisms responsible for inhibition by flavonoids of the mutagenicity of IQ in Salmonella, we investigated the effects of flavonoids on the cytosol-mediated bacterial mutagenicity of IQ. The results presented in Figs. 2 and 3 demonstrate that IQ was indeed activated to mutagenic species in a dose-dependent man-

ner by the cytosol (S105 fraction) alone, but clearly less efficiently than by the S9 fraction. Mutagenic activities were 109.6 revertants/μl S9 fraction (= 100%) and 9.76 revertants/μl S105 fraction (8.9%) (slope in the linear range) while the plateau achievable with the S105 fraction was about 37% of that of the S9 fraction. Under the test conditions with the S9 fraction employed by us, the contribution of the factors from the cytosolic S105 fraction might be about 12% (Fig. 2) provided that the activating effects of microsomes and cytosol are additive. As can be seen from Table 3, 19 out of 20 flavonoids inhibited the mutagenicity of IQ in *S. typhimurium* TA98 which is exhibited in the presence of S105. An analysis of structure–antimutagenicity relationships showed that within each subgroup of flavonoids, the respective parent compound already was a potent inhibitor of mutagenic activity of IQ. Additional

Table 1
Effects of flavonoids and related compounds on 7-methoxyresorufin-demethylase (MROD) and 7-ethoxyresorufin-deethylase (EROD) activities in liver microsomes of rats and on the mutagenicity of IQ in the Salmonella/reversion assay



Flavone Isoflavone Flavanone

Compound	Substituents	IC_{50} (μM) [a]			IC_{50}/IQ [c] (nmol/ml top agar)
		MROD (Aroclor 1254) [b]	MROD (β-naphto-flavone) [b]	EROD (Aroclor 1254) [b]	
Flavones					
Flavone		0.9	0.6	0.5	1.09
6-Hydroxyflavone	6-OH	2.0	1.3	1.9	34.4
Apigenin	5,7,4′-OH	1.3	1.0	n.d.	0.29
Luteolin	5,7,3′,4′-OH	1.2	n.d.	n.d.	0.85
Tangeretin	5,6,7,8,4′-OCH_3	0.9	0.7	0.7	2.67
Flavonols					
Flavonol	3-OH	0.4	0.6	0.4	0.67
Kaempferol	3,5,7,4′-OH	0.4	0.9	0.7	2.56
Fisetin	3,7,3′,4′-OH	n.d.	4.4	n.d.	2.35
Morin	3,5,7,2′,4′-OH	6.9	7.1	6.2	18.1
Flavanones					
Flavanone		1.1	1.5	3.6	1.47
2′-Hydroxyflavanone	2′-OH	2.4	n.d.	6.3	32.8
Hesperetin	5,7,3′-OH; 4′-OCH_3	3.0	4.0	3.8	7.2
Isoflavones					
Genistein	5,7,4′-OH	3.3	2.9	n.d.	–
Biochanin A	5,7-OH; 4′-OCH_3	8.6	n.d.	9.8	35.7
Chalcone and related compounds					
Chalcone (benzylideneacetophenone)		3.8	4.8	0.9	2.67
Benzylideneacetone		4.7	n.d.	6.6	25.3

n.d., not determined; –, inactive.
[a] IC_{50} is the concentration of a flavonoid in μM required to inhibit the activity of MROD or EROD by 50%; regressions: r^2-values ranged from 0.90 to 0.99, confidence limits for statistical analysis obtained by the Wilcoxon test; each value represents the average of three measurements.
[b] Compound used to induce MROD or EROD activities in rat liver.
[c] IC_{50}/IQ is the concentration of a flavonoid in nmol/ml top agar required to inhibit the mutagenic activity of IQ by 50%, values recalculated from [1].

hydroxyl groups either did not change much (apigenin, luteolin–flavone; kaempferol–flavonol) or reduced antimutagenic potency considerably depending on number and position of hydroxyl functions (6-hydroxflavone; fisetin, morin, robinetin, myricetin). Methylation of hydroxyl functions, however, always increased antimutagenic potency – 6-methoxyflavone, tangeretin, biochanin A, hesperetin.

In additional experiments, we used methodological variations of the Salmonella test system as de-

Table 2
Inhibition by flavonoids of mutagenic activity induced by *N*-OH-IQ [a] in *S. typhimurium* TA98 NR

Compound	Substituents [b]	IC_{50} (nmol/ml top agar)	% Inhibition at 500 nmol/plate [c]	Antimutagenic potency
Flavone			10.8	–
5-Hydroxyflavone	5-OH		5.2	–
6-Hydroxyflavone	6-OH		+32.8 [d]	[+] [e]
6-Methoxyflavone	6-OCH_3		+15.5	–
7-Hydroxyflavone	7-OH		30.1	+
Chrysin	5,7-OH		32.0	+
Apigenin	5,7,4′-OH	66.7	57.5	+ +
Luteolin	5,7,3′,4′-OH	25.0	67.4	+ + +
Diosmetin	5,7,3′-OH; 4′-OCH_3	(7.0) [f,g]	+61.0 [g]	[+ +]
Tangeretin	5,6,7,8,4′-OCH_3		4.2	–
Flavonol	3-OH		+41.1	[+]
Kaempferol	3,5,7,4′-OH		41.0	+
Kaempferol trimethylether	5-OH; 3,7,4′-OCH_3		+72.4	[+]
Fisetin	3,7,3′,4′-OH	15.0	84.5	+ + +
Isorhamnetin	3,5,7,4′-OH; 3′-OCH_3	(30.0) [f,h]	+23.1 [h]	[+ +]
Morin	3,5,7,2′,4′-OH	148	53.0	+ +
Quercetin	3,5,7,3′,4′-OH	130 [i]	57.2 [i]	+ +
Rutin	3-*O*-Rut; 5,7,3′,4′-OH		11.8	–
Robinetin	3,7,3′,4′,5′-OH	135	50.0	+ +
Myricetin	3,5,7,3′,4′,5′-OH	33.6	65.1	+ +
Myricitrin	3-*O*-Rha; 5,7,3′,4′,5′-OH		15.8	–
Genistein [j]	5,7,4′-OH		28.6	(+)
Biochanin A [j]	5,7-OH; 4′-OCH_3		34.1	+
Flavanone	2,3-H;		11.0	–
2′-Hydroxyflavanone	2,3-H; 2′-OH		+32.9	[+]
Naringenin	2,3-H; 5,7,4′-OH		28.0	(+)
Eriodictiol	2,3-H; 5,7,3′,4′-OH		+62.1	[+]
Hesperetin	2,3-H; 5,7,3′-OH;4′-OCH_3		11.3	–
Chalcone (benzylideneacetophenone)			+14.9	–
Benzylideneacetone			+36.6	[+]

IC_{50} is the concentration of a given flavonoid required to inhibit the mutagenicity of *N*-OH-IQ by 50%, calculated from corresponding dose–response curves. (+), marginal; +, weak; + +, moderate; + + +, strong antimutagenic potency; –, inactive.
[a] 1.5 ng *N*-OH-IQ produced 1026 ± 58 revertants/plate.
[b] The various structures of flavonoids subgroups are shown in Table 1.
[c] Mutagenic activity was not completely eliminated by the flavonoids, but in many cases reduced to a plateau as indicated.
[d] Numbers with the prefix + in this column represent enhancement of mutagenicity.
[e] Numbers in square brackets indicate enhancement of mutagenicity.
[f] Numbers in parentheses in this column indicate the EC_{200} value, the concentration of a flavonoid required for enhancement of mutagenic activity by 100%.
[g] Maximum of mutagenic activity + 105.1% at 13.4 nmol/ml top agar.
[h] Maximum of mutagenic activity + 123.9% at 53.4 nmol/ml top agar.
[i] After correction for mutagenic activity, see Materials and methods, Section 2.5.
[j] Isoflavonoid; *O*-Rha, *O*-rhamnose; *O*-Rut, *O*-rutinose.

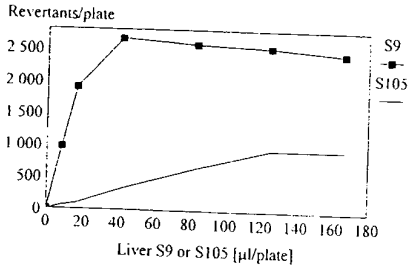


Fig. 2. Induction of his$^+$-revertants in *S. typhimurium* TA98 by IQ (25 ng/plate) in the presence of various amounts of liver S9 or S105 fractions from Aroclor 1254-treated rats. Each point represents the average of three plates, each test series was performed in duplicate. Spontaneous revertants are subtracted.

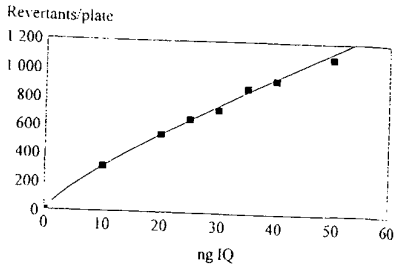


Fig. 3. Induction of his$^+$-revertants in *S. typhimurium* TA98 by various doses of IQ in the presence of liver S105 from Aroclor 1254-treated rats. Each point represents the average of three plates, each test series was performed in duplicate. Spontaneous revertants are subtracted.

Table 3
Antimutagenic effects of flavonoids on the S105-mediated mutagenicity of IQ [a] induced in *S. typhimurium* TA98

Flavonoid [b]	IC_{50} (nmol/ml top agar)	% Inhibition at 500 nmol/plate [c]	Antimutagenic potency
Flavones			
Flavone	1.89	91.1	+ + +
6-Hydroxyflavone	19.9	84.1	+
6-Methoxyflavone	2.19	88.4	+ + +
Apigenin	2.19	95.5	+ + +
Luteolin	2.64	88.4	+ + +
Tangeretin	4.61	83.5	+ + +
Flavonols			
Flavonol	2.85	90.6	+ + +
Kaempferol	2.40	92.0	+ + +
Fisetin	10.8	85.5	+ +
Morin	20.0	76.3	+
Robinetin	32.3	77.2	+
Myricetin	25.2	70.2	+
Flavanones			
Flavanone	3.55	98.2	+ + +
2′-Hydroxyflavanone	10.4	88.9	+ +
Naringenin	23.7	80.7	+
Hesperetin	6.00	80.8	+ +
Isoflavones			
Genistein	–	20.2	–
Biochanin A	17.9	80.7	+
Chalcone and related compounds			
Chalcone (benzylideneacetophenone)	5.04	88.1	+ + +
Benzylideneacetone	26.7	75.2	+

IC_{50} is the concentration of a flavonoid in nmol/ml top agar required to inhibit the mutagenic activity of IQ by 50%, calculated from corresponding dose–response curves. +, weak; + +, moderate; + + +, strong antimutagenic potency; –, inactive.

[a] 25 ng IQ/test produced 659 ± 47 revertants/plate.

[b] The various structures of flavonoids are shown in Table 1.

[c] Mutagenic activity was not completely eliminated, but reduced, as indicated.

scribed by de Flora [17] in order to evaluate other conceivable mechanisms of antimutagenicity of flavonoids. In pretreatment experiments with IQ as inducer of mutations, two of the most potent flavonoids in the standard assay, flavone and flavonol were, however, inactive.

When these experiments were repeated with *N*-OH-IQ and 9 flavonoids, luteolin and fisetin reduced revertant numbers by 34.2 and 40.6%, respectively (confidence limits, $p < 0.01$) while flavone, tangeretin, kaempferol, quercetin, flavanone, naringenin and hesperetin were inactive. This result is suggestive of a favored penetration of luteolin and fisetin into bacterial cells prior to exposure to the mutagen and an inhibition of phase II esterification reactions. In post-treatment experiments, according to the basic procedure (variation A), flavone and fisetin did not affect the number of his$^+$-revertants induced by *N*-OH-IQ in *S. typhimurium* TA98NR which means that no effects on DNA repair were detectable. This procedure, however, implies that the modulator exerts a persistent action throughout the 48-h growth period in soft agar. In variation B, the effects of flavone and fisetin were limited to a 30-min period by the experimental design. This time flavone and fisetin in concentrations of 400 nmol/test reduced revertant numbers by 19.4 and 25.6%, respectively (confidence limits, $p < 0.02$). Again, in variation C, designed to detect late effects of the modulator itself, a 12.3 and 28.9% reduction of *N*-OH-IQ induced revertant numbers by flavone and fisetin was observed, however, again only at the highest concentrations. These results suggest a weak influence of flavone and fisetin on fixation and expression of DNA damage.

4. Discussion

In previous investigations on the relationship of flavonoid structure and antimutagenicity against mutagenicity of IQ in the Salmonella/reversion assay, we had demonstrated that antimutagenicity was strictly dependent on the presence of a carbonyl group at carbon 4 of the flavane nucleus, widely dependent on the aglycone nature of the compound, quantitatively influenced by the presence of the double bond between C2 and C3, but independent of the existence of ring C [1]. In the chalcone series, ring I was not absolutely necessary for antimutagenic activity, whereas the olefinic double bond proved to be an indispensable determinant of antimutagenicity. With respect to flavones, flavonols, flavanols and flavanones, our results were confirmed by Lee et al. [27]. Within the flavone series, ID_{50} values estimated by us and by these authors were closely similar. However, in the flavanone and especially in the flavonol subgroups, quantitative divergencies were evident. The hypothesis that hydroxyl functions at carbons 5, 7 and 4′ would increase antimutagenic potency is directly opposite to our results.

With respect to the mechanisms by which flavonoids exert their antimutagenic effects against mutagenicity of IQ in *S. typhimurium* TA98 it had been hypothesized by us previously that multifactorial inhibition takes place [1]. Since IQ is metabolically activated in rat liver by the isoforms of cytochrome P-450 dependent 1A1 and 1A2 monooxygenases, reactions which may be monitored by measuring 7-methoxy-and 7-ethoxyresorufin-dealkylases, inhibition of MROD and EROD activities by flavonoids was investigated at first. In agreement with others [27–29], it could be demonstrated that flavonoids indeed inhibited these activities. In general, there was good agreement between inhibitory potencies against MROD and EROD activities and former results on the antimutagenic potencies against IQ in *S. typhimurium* as can be seen from Table 1. However, the correlation between an increasing number of hydroxyl groups and the reduction of inhibitory activities was less clearly expressed: it was observed in the case of morin and fisetin as compared with flavonol, but was absent in the flavone subgroup. Furthermore, distinct discrepancies are noteworthy. First, the interfering influence of a hydroxyl function at C6 or C2′ (6-hydroxyflavone, 2′-hydroxyflavanone, morin) in the Salmonella assay was not visible with respect to MROD and EROD activities. Again, the isoflavonoids genistein and biochanin A, the former inactive and the latter of moderate antimutagenic potency in the Salmonella assay, considerably inhibited MROD activity. On the other hand, IC_{50} values of apigenin and luteolin estimated in the Salmonella assay were consistently lower than those obtained with respect to MROD activities, suggesting addi-

tional factors influencing antimutagenicity. However, these discrepancies could not be explained by effects on NADPH-cytochrome *c* reductase activity since all flavonoids tested except chalcone were inactive.

Phase II esterification reactions of *N*-OH-IQ, the proximate mutagen of IQ, may occur in rat liver and in the bacteria used as tester organism as well. In the standard Salmonella assay, the contribution of phase II liver enzymes should be low because of considerable dilution of the necessary cofactors. Experiments presented in this study were performed in the absence of S9 and are therefore limited exclusively to the metabolism with bacterial enzymes. The results indicate that among active flavonoids, inhibition of phase II reactions does not contribute to the antimutagenicity of non-polar compounds (flavone, flavanone, chalcone, benzylideneacetone), of flavones with hydroxyl functions at carbons 3, 5 and 6, of flavonoids with methoxyl functions (tangeretin, diosmetin, kaempferoltrimethylether, isorhamnetin, hesperetin) and of all hydroxylated flavanones. A contribution to the antimutagenic effects of polyhydroxylated flavones and flavonols seems possible and might, at least in part, explain the higher antimutagenic potency of myricetin as compared with robinetin and the closely similar ID_{50} values of kaempferol and fisetin in the Salmonella assay despite distinct differences in inhibitory potency against MROD activity. On the other than, IC_{50} values about 60 and 20 times higher for apigenin and luteolin question a major contribution of inhibition of esterification reactions to the reduction of antimutagenic potency in the overall reaction as compared with inhibition of MROD activity. Other influences, such as interactions with biological membranes and effects on expression and fixation of DNA damage, may also be at work as indicated by the results of pre- and post-treatment experiments designed according to de Flora et al. [17]. No explanation can be derived from these investigations with respect to the inactivity of genistein and the low activity of biochanin A in the Salmonella assay, nor can the weak comutagenic activities exerted by 6-hydroxflavone, 2′-hydroxyflavanone, and benzylideneacetone explain major decreases of antimutagenic potencies of these compounds. However, the slight increase of the ID_{50} value from luteolin to diosmetin from 3.2 to 6 nmol/plate [1], contradictory to the general rule of an increase of antimutagenic potency with methylation of hydroxyl functions, may be generated by the potent comutagenic effect of the latter compound with respect to *N*-OH-IQ induced mutagenesis.

Besides metabolic activation of heterocyclic amines via *N*-hydroxylation by membrane-bound cytochrome P-450 dependent monoxygenases cytosolic activation to mutagenic metabolites has been described by several authors [30–33] and is thought to be performed by DT diaphorase [33]. In the present study, we indeed detected a contribution of this type of activation mediated by the S105 fraction which was effectively inhibited by different flavonoids. The structure–antimutagenicity pattern was quite similar to that obtained when the postmitochondrial fraction was used and so were IC_{50} values of many flavonoids. Again, 6-hydroxyflavone, morin, robinetin, myricetin, 2′-hydroxyflavanone and benzylideneacetone were less effective antimutagens than the related more non-polar compounds. In part, this was also true in the case of the S9 activation. The only exceptions among 20 compounds were fisetin and naringenin, which were distinctly less effective in this system. Since the antimutagenic effect of the cytosolic activation cannot be explained by the inhibition of cytochrome P-450-dependent monooxygenases, inhibition of DT diaphorase should be considered. Regarding this, it could be worthwhile to isolate DT diaphorase and to find out whether with the purified enzyme an identical pattern of inhibition will be observed. So far, the remarkable similarities obtained with the S9 and S105 fractions are surprising since cytosolic activation should amount to only about 10% of that obtained with the complete activation system consisting of microsomal and cytosolic fractions. The presence of additional modifying factors might be responsible for this observation because enhancing effects of the S105 fractions on mutagenicity of aromatic and heterocyclic amines in *Salmonella typhimurium* have already been described [34,35]. Although further experiments are needed to elucidate all mechanisms participating in inhibition by flavonoids of mutagenicity of IQ in *Salmonella typhimurium* results obtained in this study demonstrate a dual role for flavonoids because they not only inhibit membrane-bound cytochrome P-450-dependent monooxygenases, but also inhibit various soluble enzymatic factors and suggest interactions with biological membranes and effects on expression and fixation of DNA damage.

References

[1] R. Edenharder, I. von Petersdorff, R. Rauscher, Antimutagenic effects of flavonoids, chalcones and structurally related compounds on the activity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and other heterocyclic amine mutagens from cooked food, Mutation Res. 287 (1993) 261–274.

[2] J.S. Felton, M.C. Knize, Occurrence, identification and bacterial mutagenicity of heterocyclic amines in cooked food, Mutation Res. 259 (1991) 205–217.

[3] K. Skog, Cooking procedures and food mutagens: a literature review, Food Chem. Toxicol. 31 (1993) 655–675.

[4] D.W. Layton, K.T. Bogen, M.G. Knize, F.T. Hatch, V.M. Johnson, J.S. Felton, Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research, Carcinogenesis 16 (1995) 39–52.

[5] Sugimura, T., S. Sato, H. Ohgaki, S. Takayama, M. Nagao, K. Wakabayashi, Overview: mutagens and carcinogens in cooked food, in: I. Knudsen (Ed.), Genetic Toxicology of the Diet. Prog. Clin. Biol. Res., Liss, New York, 1986, pp. 85–106.

[6] H. Ohgaki, S. Takayama, T. Sugimura, Carcinogenicities of heterocyclic amines in cooked food, Mutation Res. 259 (1991) 399–410.

[7] IARC, Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation, IARC Monogr. 40 (1986) 223–228.

[8] F.T. Hatch, M.G. Knize, D.H. Moore, J.S. Felton, Quantitative correlation of mutagenic and carcinogenic potencies for heterocyclic amines from cooked foods and additional aromatic amines, Mutation Res. 271 (1992) 269–287.

[9] K.A. Steinmetz, J.D. Potter, Vegetables, fruits and cancer. I. Epidemiology, Cancer Causes Control 2 (1991) 325–357.

[10] K.A. Steinmetz, J.D. Potter, Vegetables, fruits and cancer. II. Mechanisms, Cancer Causes Control 2 (1991) 427–442.

[11] G. Block, B. Patterson, A. Subar, Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence, Nutr. Cancer 18 (1992) 1–29.

[12] H. Hayatsu, S. Arimoto, T. Negishi, Dietary inhibitors of mutagenesis and carcinogenesis, Mutation Res. 202 (1988) 429–446.

[13] E. Hartman, D.M. Shankel, Antimutagens and anticarcinogens: a survey of putative interceptor molecules, Environ. Mol. Mutagen. 15 (1990) 145–182.

[14] M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, D. Kromhout, Intake of potentially anticarcinogenic flavonoids and their determinants in adult in the Netherlands, Nutr. Cancer 20 (1993) 21–29.

[15] B. Stavric, T.I. Matula, Biological significance of flavonoids in foods: a review of some recent issues and problems, Bull. Liaison-Groupe Polyphenols 14 (1988) 95–104.

[16] B. Stavric, Role of chemopreventers in human diet, Clin. Biochem. 27 (1994) 319–332.

[17] S. de Flora, A. Camoirano, F. D'Agostini, R. Balansky, Modulation of the mutagenic response in prokaryotes, Mutation Res. 267 (1992) 183–192.

[18] R. Edenharder, C. Speth, M. Decker, H. Kolodziej, O. Kayser, K.L. Platt, Inhibition of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) by coumarins and furanocoumarins, chromanones and furanochromanones, Mutation Res. 345 (1995) 57–71.

[19] B.M. Elliott, R.D. Combes, C.R. Elcombe, D.G. Gatehouse, G.G. Gibson, J.M. Mackay, R.C. Wolf, Alternatives to Aroclor 1254-induced S9 in in vitro genotoxicity assays, Mutagenesis 7 (1992) 175–177.

[20] D.M. Maron, B.N. Ames, Revised methods for Salmonella mutagenicity test, Mutation Res. 113 (1983) 173–215.

[21] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol Chem. 193 (1951) 265–276.

[22] T.C. Pederson, J.A. Buege, S.D. Aust, Microsomal electron transport: the role of reduced nicotinamid adenine dinucleotide phosphate-cytochrome c reductase in liver microsomal lipid peroxidation, J. Biol. Chem. 248 (1973) 7134–7141.

[23] M.D. Burke, R.T. Mayer, Ethoxyresorufin: direct fluorimetric assay of microsomal O-dealkylation which preferentially induced by methylcholanthrene, Drug Metab. Disp. 2 (1984) 243–249.

[24] N.S. Waleh, S.J. Rapport, K. Mortelmans, Development of a toxicity test to be coupled to the Ames Salmonella assay and the method of construction of the required strains, Mutation Res. 97 (1982) 247–256.

[25] M. Nagao, N. Morita, T. Yahagi, M. Shimizu, M. Kuroyanagi, M. Fukuoka, K. Yoshihira, S. Natori, T. Fujino, T. Sugimura, Mutagenicities of 61 flavonoids and 11 related compounds, Environ. Mutagen. 3 (1981) 401–419.

[26] H. Czeczot, B. Tudek, J. Kusztelak, T. Szymezyk, B. Dobrowolska, G. Glinkowska, J. Malinowski, H. Strzelecka, Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs, Mutation Res. 240 (1990) 209–216.

[27] H. Lee, H.W. Wang, H.Y. Su, N.J. Hao, The structure–activity relationships of flavonoids as inhibitors of cytochrome P-450 enzymes in rat liver microsomes and the mutagenicity of 2-amino-3-methyl-imidazo [4,5-*f*] quinoline, Mutagenesis 9 (1994) 101–106.

[28] R.L. Sousa, M.A. Marletta, Inhibition of cytochrome P-450 activity in rat liver microsomes by the naturally occurring flavonoid quercetin, Arch. Biochem. Biophys. 140 (1985) 345–357.

[29] M.-H. Siess, A.-M. le Bon, M. Suschetet, P. Rat, Inhibition of ethoxyresorufin deethylase activity by natural flavonoids in human and rat liver microsomes, Food Addit. Contam. 7 (Suppl. 1) (1990) 178–181.

[30] R. Forster, M.H.L. Green, A. Priestley, J.W. Gorrod, Apparent activation of 2-acetylaminofluorene and some aromatic amines by cytosolic preparations, Mutation Res. 85 (1981) 187–194.